

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
29 November 2001 (29.11.2001)

PCT

(10) International Publication Number
WO 01/90372 A1

(51) International Patent Classification⁷: C12N 15/54,
9/10, 1/21, C12P 19/18, C08B 37/00, A23C 9/123 //
(C12N 9/10, C12R 1:225)

Amersfoort (NL). LEER, Robert, Jan [NL/NL]; Kompas
7, NL-3904 PN Veenendaal (NL).

(21) International Application Number: PCT/NL01/00393

(74) Agent: JORRITSMA, Ruurd; Nederlandsch Octrooi-
bureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS
The Hague (NL).

(22) International Filing Date: 23 May 2001 (23.05.2001)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
00201871.1 25 May 2000 (25.05.2000) EP

(71) Applicant (*for all designated States except US*): NED-
ERLANDSE ORGANISATIE VOOR TOEGEPAST-
NATUURWETENSCHAPPELIJK ONDERZOEK
TNO [NL/NL]; Schoemakerstraat 97, NL-2628 VK Delft
(NL).

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): VAN GEEL
SCHUTTEN, Gerritdina, Hendrika [NL/NL]; Bosstraat
78, NL-3971 XG Driebergen (NL). DIJKHUIZEN, Lub-
bert [NL/NL]; Ter Borch 28, NL-9472 RB Zuidlaren (NL).
RAHAOUI, Hakim [NL/NL]; Sextant 4 b, NL-3813 VS

Published:

— with international search report

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: LACTOBACILLUS REUTERI GLUCOSYLTRANSFERASE

(57) Abstract: The present invention describes a protein having glucosyltransferase activity. This protein is derived from lactobacilli, which are food-grade microorganisms with the Generally Recognised As Safe (GRAS) status. The protein produces a glucan with a unique structure having 4-linked, 6-linked and 4,6-linked anhydroglucose units or in the presence of suitable acceptors, oligosaccharides. According to the invention lactobacilli capable of producing this glucan using the novel glucosyltransferase can be used as a probiotic or synbiotic.

WO 01/90372 A1

LACTOBACILLUS REUTERI GLUCOSYLTRANSFERASE

[0001] The present invention is in the field of enzymatic production of biomolecules. The invention is particularly concerned with a novel type of glucosyltransferase derived from lactobacilli and with a process for recombinant production of the enzyme and for the
5 production of useful glucans and gluco-oligosaccharides from sucrose.

Background of the invention

[0002] Lactic acid bacteria (LAB) play an important role in the fermentative production of food and feed. Traditionally, these bacteria have been used for the production of for instance wine, beer, bread, cheese and yoghurt, and for the preservation of food and feed,
10 e.g. olives, pickles, sausages, sauerkraut and silage. Because of these traditional applications, lactic acid bacteria are food-grade micro-organisms that possess the Generally Recognised As Safe (GRAS) status. Due to the different products which are formed during fermentation with lactic acid bacteria, these bacteria contribute positively to the taste, smell and preservation of the final product. The group of lactic acid bacteria includes
15 several genera such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, etc.

[0003] In recent years also the health promoting properties of lactic acid bacteria have received much attention. They produce an abundant variety of exopolysaccharides (EPS's). These polysaccharides are thought to contribute to human health by acting as prebiotic substrates, nutraceuticals, cholesterol lowering agents or immunomodulants.

[0004] To date high molecular weight polysaccharides produced by plants (such as cellulose, starch and pectin), seaweeds (such as alginate and carrageenan) and bacteria (such as alginate, gellan and xanthan) are used in several industrial applications as viscosifying, stabilising, emulsifying, gelling or water binding agents. Although all these polysaccharides are used as food additives, they originate from organisms not having the
20 GRAS status. Thus they are less desirable than the exopolysaccharides of micro-organisms, such as lactic acid bacteria, which have the GRAS status.

[0005] The exopolysaccharides produced by lactic acid bacteria can be divided in two groups, heteropolysaccharides and homopolysaccharides; these are synthesised by totally different mechanisms. The former consist of repeating units in which residues of different
30 types of sugars are present and the latter consist of one type of monosaccharide. The synthesis of heteropolysaccharides by lactic acid bacteria, including lactobacilli, has been studied extensively in recent years. Considerable less information is present on the synthesis of homopolysaccharides from lactobacilli, although some studies have been

performed. The information on the synthesis of homopolysaccharides in lactobacilli is mainly limited to the synthesis of glucans and only two reports, written by the present inventors, exist on the synthesis of fructans. In one of these reports the *Lactobacillus reuteri* strain LB 121 was found to produce both a glucan and a fructan when grown on sucrose, but only a fructan when grown on raffinose (van Geel-Schutten, G.H. *et al.*, Appl. Microbiol. Biotechnol. (1998) 50, 697-703). In the other report was found that *Lactobacillus reuteri* strain LB 35-5, a spontaneous mutant of *Lactobacillus reuteri* strain LB 121, only produced a glucan when grown on sucrose (van Geel-Schutten, G.H. *et al.*, Appl. Environ. Microbiol. (1999) 65, 3008-3014). In the other report the soluble glucan and fructan were also characterised by their molecular weights (of 3,500 and 150 kDa respectively) and the glucan was reported to be highly branched with a unique structure consisting of a terminal, 4-substituted, 6-substituted, and 4,6-di-substituted α -glucose in a molar ratio 1.1 : 2.7 : 1.5 : 1.0 (van Geel-Schutten, G.H. *et al.*, Appl. Environ. Microbiol. (1999) 65, 3008-3014). No structural identification of a similar glucan produced by a *Lactobacillus* had been reported before. The fructan was identified as a (2 \rightarrow 6)- β -D-fructofuranan (also called a levan). This was the first example of levan synthesis by a *Lactobacillus* species.

Summary of the invention

[0006] A novel enzyme having glucosyltransferase activity using sucrose as a substrate has now been found in *Lactobacillus reuteri*, and its amino acid sequence and other structural properties have been determined. The enzyme is unique in that it is capable of producing a highly branched glucan with α -1,4- and α -1,6-glucosidic links. The invention thus pertains to an enzyme, to DNA encoding it, to cells containing such DNA and to their use in producing carbohydrates, as defined in the appending claims. The invention also pertains to glucans so produced as well as to oligosaccharides and chemically derivatised glucans.

Description of the invention

[0007] It was found according to the invention that the glucans are produced by certain *Lactobacillus* strains, in particular by certain strains of *Lactobacillus reuteri*, as a result of the activity of a single glucosyltransferase (glucansucrase).

[0008] The nucleotide and amino acid sequence of the novel glucosyltransferase are represented by SEQ ID No. 1 and 2, respectively, and are also shown in figure 3. The start codon of the glucosyltransferase is preceded by a putative ribosome binding site with the

nucleotide sequence GAAGGAGA (located 5 base pairs upstream of the start codon of the glucosyltransferase; see SEQ ID No. 1 and figure 3). Furthermore, the start codon is preceded by the nucleotide sequence TATAAT, also called Pribnow box or -10 region, (located 42 base pairs upstream of the start codon; see SEQ ID No. 1 and figure 3) and by the nucleotide sequence TTGAAA, also called -35 region (located 80 base pairs upstream of the start codon; see SEQ ID No. 1 and figure 3).

[0009] The invention covers a protein having glucosyltransferase activity with sucrose as substrate comprising an amino acid sequence with an amino acid identity of at least 50%, preferably at least 60%, and more preferably at least 70%, compared to the amino acid sequence 531-1781 of SEQ ID No. 2. The invention also covers a part of a protein with at least 15 contiguous amino acids which are identical to the corresponding part of the amino acid sequence 531-1781 of SEQ ID No. 2. The present invention covers a protein having glucosyltransferase activity with sucrose as donor substrate with an amino acid identity of at least 50%, preferably at least 60%, and more preferably at least 70%, compared to the amino acid sequence of SEQ ID No. 2. The invention also covers a part of a protein with at least 15 contiguous amino acids which are identical to the corresponding part of the amino acid sequence of SEQ ID No. 2. The novel glucosyltransferase has homology with several other proteins as revealed by amino acid sequence alignment. A high homology (figure 5) was found with an alternansucrase of *Leuconostoc mesenteroides* strain NRRL B-1355 (46% identity, within 1261 amino acids of amino acid sequence 531-1781 of SEQ ID No. 2) and a dextransucrase of *Leuconostoc mesenteroides* strain NRRL B-512F (44% identity, within 1270 amino acids of amino acid sequence 531-1781 of SEQ ID No. 2). Furthermore, the alignment revealed the presence of various domains also found in the other glucosyltransferases, such as an N-terminal variable domain, a catalytic domain and a C-terminal glucan binding domain. The N-terminal domain shows almost no identity with the N-terminal domains of other glucosyltransferases and an N-terminal signal peptide could not be detected.

[0010] The invention also covers a protein comprising an amino acid sequence of at least 100 amino acids, exhibiting at least 55%, preferably at least 65% amino acid identity with the corresponding part of the amino acid sequence 972-1514 (catalytic domain) of SEQ ID No. 2. The catalytic domain shows a high level of homology (about 50% identity) with other known streptococcal and *Leuconostoc* glucosyltransferases and putative functions based on the alignment can be ascribed to several amino acids within this catalytic domain (figure 4). Asp-1024, Glu-1061 and Asp-1133 of SEQ ID No. 2 are putative catalytic

residues, Asp-984 of SEQ ID No. 2 is a putative calcium binding residue and Arg-1022 of SEQ ID No. 2 a putative chloride binding residue. His-1132 and Gln-1514 of SEQ ID No. 2 may stabilise the transition state and the residues Asp-1027, Asn-1028, Asp-1062 and Trp-1063 of SEQ ID No. 2 may play a role in binding of acceptor molecules and in the transfer of the glucosyl moiety.

[0011] The invention further covers a protein comprising an amino acid sequence of at least 100 amino acids, exhibiting at least 50%, preferably at least 60%, amino acid identity with the corresponding part of the amino acid sequence 1515-1781 (glucan binding domain) of SEQ ID No. 2. The C-terminal putative glucan binding domain is much shorter than the corresponding domains in other glucosyltransferases but three known repeats, resembling YG repeats, are described: YYFYDLAGNMVKN (SEQ ID No. 3) starting at amino acid position 1656 of SEQ ID No. 2, WYFFDQDGKMVEN (SEQ ID No. 4) starting at amino acid position 1678 of SEQ ID No. 2 and TYYFDNYGKMVRN (SEQ ID No. 5) starting at amino acid position 1725 of SEQ ID No. 2. YG repeats are defined by the presence of one or more aromatic residues (of which one is usually tyrosine), followed by 3-4 glycine residues downstream, a hydrophobic residue, a neutral polar residue (usually glycine or asparagine) and 1-3 hydrophobic residues. It is striking that the number of repeats necessary to ensure glucan binding properties is different for enzymes producing a soluble or an insoluble glucan. Possibly the glucan binding domain is also involved in the determination of the glucan structure and the polymer chain growth. Furthermore, this domain seems also necessary for the complete glucosyltransferase activity.

[0012] Specific amino acids of the glucosyltransferase that are believed to be important for the unique properties of the enzyme include Pro-1026, Ile-1029, Met-1034, Asn-1035, Ser-1136, Ala-1143, Ile-1170, Leu-1223, Ala-1413, Val-1418, Ala-1428, Leu-1442 of the amino acid sequence of SEQ ID No. 2. So a protein, mutant or part thereof, comprising at least one of the above mentioned amino acids is also part of the invention. Particularly Pro-1026 and Ile-1029 are of interest. Pro-1026 is found in a position where a conserved Val is found in other glucosyltransferases. Compared to Val, the presence of Pro results in a more rigid protein structure. This change of protein structure may influence the glucosidic bonds formed and might explain the unique structure of the glucan. Ile-1029 is also found in a position where a conserved Val is present in other LAB glucosyltransferases not producing $\alpha(1,4)$ bonds. An identical amino acid substitution is observed in amylosucrase, a glucosyltransferase synthesising $\alpha(1,4)$ bonds.

[0013] A nucleotide sequence encoding any of the above mentioned proteins, mutants, variants or parts thereof is also a subject of the invention. Furthermore, the nucleic acid sequences corresponding to expression-regulating regions (promoters, enhancers, terminators) contained in the nucleic acid sequence (1)-(160) or (5507)-(6026) of SEQ ID No. 1 (see also figure 3) can be used for homologous or heterologous expression of genes. Such expression-regulating sequences are operationally linked to a polypeptide-encoding nucleic acid sequence such as the genes of the glucosyltransferase according to the invention. Inverted repeats are located 62 base pairs downstream the termination codon (TAA), suggesting the presence of a Rho independent transcription termination signal. The -10 and -35 consensus promoter sequences, two motifs generally present upstream of the start codon of procaryotes, are identified as described above. Other promoter, enhancer or terminator were not identified. A nucleic acid construct comprising the nucleotide sequence operationally linked to an expression-regulating nucleic acid sequence is also covered by the invention.

[0014] A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. A part of the gene encoding the N-terminal part, the catalytic domain and the glucan-binding domain of the glucosyltransferase has been cloned and expressed in *E. coli*. The molecular weight predicted from the deduced amino acid sequence of that part (*i.e.* the amino acid sequence 531-1781 of SEQ ID No. 2; see examples) of the recombinant glucansucrase expressed in *E. coli* is 145 kDa.

[0015] The invention further covers a enzymatically active protein as defined above which, in the presence of sucrose, produces a glucan having 38-48% 4-linked anhydroglucose units, 17-28% 6-linked anhydroglucose units, and 7-20% 4,6-linked anhydroglucose units, preferably a glucan having 40-46% 4-linked anhydroglucose units, 19-26% 6-linked anhydroglucose units, and 9-18% 4,6-linked anhydroglucose units. There is a large variation in glucans due to differences in the type of bonds present, degree and type of branching, length of the glucan chains, molecular weight, and the conformation of the polymers. The structure of this glucan is unique in that it is highly branched, consists of terminal, 4-substituted, 6-substituted, and 4,6-di-substituted α -glucose in a molar ratio 1.1 : 2.7 : 1.5 : 1.0 and has a high molecular weight of 3500 kDa. The novel glucan may be synthesised by a glucosyltransferase present in the *Lactobacillus* strains, preferably *Lactobacillus reuteri* strains and more preferably *Lactobacillus reuteri* strains LB 121 and

LB 35-5. *Lactobacillus reuteri* belongs to the group of lactic acid bacteria which are known to play an important role in the fermentative production of food and feed. Because of this, lactic acid bacteria are food-grade micro-organisms that possess the Generally Recognised As Safe (GRAS) status.

5 [0016] The invention also pertains to a process of producing a glucan as described above using an isolated glucosyltransferase according to the invention and a suitable glucose source such as for instance sucrose. The glucosyltransferase may be isolated by conventional means from the culture of a glucosyltransferase-positive lactic acid bacterium, especially a *Lactobacillus reuteri*, or from a recombinant organism expressing
10 the glucosyltransferase gene.

[0017] Additionally, the invention concerns a process of producing gluco-oligosaccharides containing the characteristic structure of the glucan described above using an isolated glucosyltransferase according to the invention. There is a growing interest in oligosaccharides derived from homopolysaccharides, for instance for prebiotic purposes.
15 Several fructo- and gluco-oligosaccharides are known to stimulate the growth of bifidobacteria in the human colon. Gluco-oligosaccharides produced by the glucosyltransferase described above can be used as prebiotics and probiotics and are also part of the invention. The production of the gluco-oligosaccharides is different from the glucan synthesis reaction. In addition to sucrose, the substrate of the glucosyltransferase, an
20 acceptor molecule such as maltose or lactose is necessary for the acceptor reaction. Another way of producing gluco-oligosaccharides is by hydrolysis of the glucan described above. This hydrolysis can be performed by known hydrolysis methods such as enzymatic hydrolysis with enzymes such as amylase, dextranase or pullulanase or by acid hydrolysis. The produced gluco-oligosaccharides must contain at least one 1,6- or one 4,6- glucosidic
25 link to be used as prebiotics.

[0018] The invention also concerns chemically modified glucans and gluco-oligosaccharides based on the 1,4/1,6- α -glucans described above. Chemical modification can be achieved by oxidation, such as hypochlorite oxidation resulting in ring-opened 2,3-dicarboxy-anhydroglucose units (see e.g. EP-A-427349), periodate oxidation resulting in
30 ring-opened 2,3-dialdehyde-anhydroglucose units (see e.g. WO 95/12619), which can be further oxidised to (partly) carboxylated units (see e.g. WO 00/26257), TEMPO-mediated oxidation resulting in 6-carboxy-anhydroglucose units (see e.g. WO 95/07303). The oxidised glucans have improved water-solubility, altered viscosity and a retarded fermentability and can be used as metal-complexing agents, detergent additives,

strengthening additives, bioactive carbohydrates, emulsifiers and water binding agents. They can also be used as starting materials for further derivatisation such as cross-linking and the introduction of hydrophobes. Oxidised glucans coupled to proteins can be used as emulsifiers and stabilisers. (Partial) hydrolysis of glucans according to the invention
5 and/or modified glucans results in gluco-oligosaccharides, which can be used as bioactive carbohydrates or prebiotics. The oxidised glucans of the invention preferably contain 0.05-1.0 carboxyl groups per anhydroglucose unit, *e.g.* as 6-carboxyl units.

[0019] Another type of chemical modification is phosphorylation, as described in O.B. Wurzburg (1986), *Modified Starches: properties and uses*. CRC Press Inc., Boca Raton,
10 97-112. One way to achieve this modification is by dry heating glucans with a mixture of monosodium and disodium hydrogen phosphate or with tripolyphosphate. The phosphorylated glucans are suitable as wet-end additives in papermaking, as binders in paper coating compositions, as warp sizing-agents, and as core binders for sand molds for metal casting. A further type of derivatisation of the glucans is acylation, especially
15 acetylation using acetic or propionic anhydride, resulting in products suitable as bleaching assistants and for the use in foils. Acylation with *e.g.* alkenyl succinic anhydrides or (activated) fatty acids results in surface-active products suitable as *e.g.* surfactants, emulsifiers, and stabilisers.

[0020] Hydroxyalkylation, carboxymethylation, and amino-alkylation are other methods
20 of chemical derivatisation of the glucans. Hydroxyalkylation is commonly performed by base-catalysed reaction with alkylene oxides, such as ethylene oxide, propylene oxide or epichlorohydrine; the hydroxyalkylated products have improved solubility and viscosity characteristics. Carboxymethylation is achieved by reaction of the glucans with mono-chloroacetic acid or its alkali metal salts and results in anionic polymers suitable for
25 various purposes including crystallisation inhibitors, and metal complexants. Amino-alkylation can be achieved by reaction of the glucans with alkylene-imines, halo-alkyl amines or amino-alkylene oxides, or by reaction of epichlorohydrine adducts of the glucans with suitable amines. These products can be used as cationic polymers in a variety of applications, especially as a wet-end additive in paper making to increase strength, for
30 filler and fines retention, and to improve the drainage rate of paper pulp. Other potential applications include textile sizing and wastewater purification. The above mentioned modifications can be used either separately or in combination depending on the desired product. Furthermore, the degree of chemical modification is variable and depends on the intended use. If necessary 100% modification, *i.e.* modification of all anhydroglucose

units can be performed. However, partial modification, e.g. from 1 modified anhydroglucose unit per 100 up to higher levels, will often be sufficient in order to obtain the desired effect.

[0021] The use of a *Lactobacillus* strain capable of producing the novel and unique glucan is also covered by the invention. Preferably, the strain is also capable of producing a fructan, which can be either a levan, inulin or both. More preferably, the strain is also capable of producing fructo-oligosaccharides. For producing this glucan and/or fructan the strain according to the invention preferably uses sucrose as a substrate. The glucan according to the invention can be produced when the strain is cultured in the presence of sucrose and/or after the strain was cultured in the presence of sucrose during a sufficient time period. The *Lactobacillus* strains used have been deposited at the BCCM/LMG Culture collection (Gent, BE) under the deposit numbers LMG P-18388 (*L. reuteri* wild-type strain LB 121) and LMG P-18389 (*L. reuteri* mutant strain LB 35-5). The efficacy of some *L. reuteri* strains as a probiotic has been demonstrated in various animals such as for instance poultry and humans. The administration of *L. reuteri* to pigs resulted in significantly lower serum total and LDL-cholesterol levels, while in children *L. reuteri* is used as a therapeutic agent against acute diarrhea. For this and other reasons *L. reuteri* has already been supplemented to commercially available probiotic products. The mode of action of *L. reuteri* as a probiotic is still unclear. Preliminary studies indicated that gut colonisation by *L. reuteri* may be of importance. According to the invention, it was found that the mode of action of *L. reuteri* as a probiotic may reside partly in the ability to produce polysaccharides. *Lactobacillus* strains, preferably *L. reuteri* strains, more preferably *L. reuteri* strains LB 121, LB 35-5 and other strains capable of producing a glucan having 38-48% 4-linked anhydroglucose units (AGU), 17-28% 6-linked AGU, and 7-20% 4,6-linked AGU, preferably a glucan having 40-46% 4-linked AGU, 19-26% 6-linked AGU, and 9-18% 4,6-linked AGU can thus advantageously be used as a probiotic. They can also, together with these polysaccharides, be used as a symbiotic.

Examples

Example 1: Isolation of DNA from *Lactobacillus reuteri*, nucleotide sequence analysis of the glucosyltransferase gene, construction of plasmids for expression of the glucosyltransferase gene in *E. coli* DH5 α , expression of an essential part of the glucosyltransferase gene in *E. coli* DH5 α , and identification of the novel glucan produced by the recombinant enzyme.

General procedures for cloning, DNA manipulations and agarose gel electrophoresis were essentially as described by Sambrook *et al.* (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York. Restriction endonuclease digestions and ligations with T4 DNA ligase were performed as recommended by the suppliers. DNA was amplified by PCR techniques using ampliTAQ DNA polymerase (Perkin Elmer). DNA fragments were isolated from agarose gels using the Qiagen extraction kit (Qiagen GMBH), following the instructions of the suppliers. *Lactobacillus reuteri* strains were grown anaerobically at 37°C in MRS medium (DIFCO) or in MRS-s medium (MRS medium containing 100 g/l sucrose instead of 20 g/l glucose) and *E. coli* strains were grown aerobically at 37°C in LB medium containing 100 µg/ml ampicillin (when appropriate 40 µg/ml X-gal was added).

For the isolation of chromosomal DNA, *L. reuteri* 121 was grown overnight at 37°C in MRS broth (Difco) supplemented with 40 mM DL-threonine. Cells of 4 ml culture were harvested by centrifugation and resuspended in 10 ml MRS broth supplemented with 40 mM DL-threonine and incubated for 2 h at 37°C. After centrifugation the cells were resuspended in 400 µl protoplast buffer (10 mM sodium maleate, pH 6.1 supplemented with 0.3 M lactose, 10 mM MgCl₂, 12% polyethylene-glycol 2000, 0.1 M EDTA, 5 mg/ml lysozyme (47,000 U/mg) and 10 U/ml mutanolysine) and incubated for 1 h at 37°C. After centrifugation (1 min, Eppendorf centrifuge), protoplasts were resuspended in 500 µl 20 mM Tris-HCl, pH 8.0. Subsequently, 100 µl laurylsarcosine and 150 µl 5 M NaCl were added and DNA was extracted. Plasmid DNA of *Lactobacillus reuteri* was isolated using a modification of the methods of Anderson and Mc Kay (1983) Appl. Environ. Microbiol. 46, 549-552 and Burger and Dicks (1994) Biotechnol. Technol. 8, 769-772. Fresh prewarmed (37°C) MRS broth (10 ml) was inoculated with 200 µl of an overnight culture and incubated for 2.5 h at 37°C. Cells were harvested by centrifugation and washed with 2 ml sterile STE buffer (0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8). After centrifugation, the pellet was resuspended in 380 µl solution I (0.5 M sucrose, 50 mM Tris-HCl, 1 mM EDTA, pH 8, containing 2 mg/ml lysozyme and 6.6 U mutanolysin). After an incubation of 1.5 h at 37°C, 50 µl of solution II (50 mM Tris-HCl, pH 8.0, 0.25 M EDTA) and 30 µl of solution III (50 mM Tris-HCl, pH 8, 20 mM EDTA, 20% SDS) were added and the suspension was mixed. Sodium hydroxide (30 µl of a 3 M solution) was added, followed by 50 µl 2 M Tris-HCl and 72 µl

5 N NaCl. After extraction with equal volumes of phenol and chloroform, the DNA was precipitated with ethanol.

The glucosyltransferase (*gtfA*) gene was identified by amplification with PCR using degenerated primers (GTFpr1, 5'GAYAAKWSNAAKSYNRTNGTNSARGC3' (SEQ ID No. 6) and GTFpr2, 5'GNKCNCANATRATRCCNCTRNA3' (SEQ ID No. 7); Y=T or C, K=G or T, W=A or T, S=C or G, R=A or G, N= A, C, G, or T) based on conserved amino acid sequences deduced from different glucosyltransferase genes (*gtfS* of *Streptococcus downei*, *gtfC* of *S. mutans*, *gtfI* of *S. downei*, *gtfK* and *gtfM* of *S. salivarius* and *dsrA* of *Leuconostoc mesenteroides*) and *Lactobacillus reuteri* chromosomal DNA as template. An amplification product with the predicted size of about 660 bp was obtained (figure 1A). To investigate the possible presence of multiple copies of the glucosyltransferase gene, Southern hybridisation was performed. DNA was restricted with endonucleases, separated by agarose gel electrophoresis and transferred to a Hybond nylon membrane. For hybridisation, probes were labelled with [α -³²P]dCTP using Random Primed DNA labelling kit (Boehringer Mannheim), following the manufacturer's instructions. The Southern hybridisation of chromosomal DNA of the *Lactobacillus reuteri* strain 121 with the amplified 660 bp PCR fragment, followed by washing under non-stringent conditions (45°C, 0.5x SSC/0.1 SDS) revealed one hybridising fragment, suggesting the presence of only a single copy of a glucosyltransferase gene in the *L. reuteri* strains. The 660 bp fragment was cloned in *E. coli* JM109 using the pCR2.1 vector. Transformations were performed by electroporation using the BioRad gene pulser apparatus at 2.5 kV, 25 μ F and 200 Ω , following the instructions of the manufacturer. The fragment was sequenced by the method of Sanger *et al.* (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467, confirming that the correct part of the *gtfA* gene had been isolated. The 660 bp amplified fragment was used to design primers for inverse PCR. Using inverse PCR techniques a 3 kb fragment of the isolated *gtfA* gene was generated (figure 1B). This 3 kb amplicon was identified by sequencing and probes were designed to isolate the *EcoRI/BglII* and *EcoRI/HindIII* fragments from a partial DNA library of *L. reuteri* in *E. coli* DH5 α (figure 1C). Positive clones were selected by colony blot hybridisation using Hybond-N filters, following the instructions of the supplier and the cloned fragments were sequenced. Attempts to clone the C-terminal part of the glucansucrase gene in *E. coli* DH5 α or JM109 using a partial DNA library strategy with different vectors failed. Therefore, the C-terminal part was isolated by inverse PCR. The remaining fragment, located between the *EcoRI/BglII* and *EcoRI/HindIII* fragments, was isolated by PCR

techniques (figure 1D). The amplicons obtained were sequenced directly. To eliminate errors due to the PCR reaction, these fragments were sequenced for at least 4 times, using different clones per PCR reaction. Both DNA strands of the entire glucosyltransferase gene were sequenced twice. In this way the sequence of a 5.5 kb region of the
5 *Lactobacillus reuteri* chromosomal DNA, containing the *gtfA* gene and its surroundings, were obtained.

The plasmids for expression of the glucosyltransferase gene in *E. coli* DH5 α were constructed as described hereafter. A 4.8 kb fragment, containing the entire glucosyltransferase gene was generated by PCR, using the primers GTFpr3 (5'ACAACCACCA
10 TGGAAATTAGG TCGCACTGAT GTAAC3') (SEQ ID No. 8) and GTFpr4 (5'GCCAGCTGGA TCCGTCGACT AGTTTATTTT TGATCAAGCA TCTTACC3') (SEQ ID No. 9). Both primers contained suitable restriction enzyme recognition sites at their 5' ends (*Nco*I in GTFpr3 and *Bam*HI and *Sa*II in GTFpr4). Cloning of this PCR fragment in different vectors failed. Therefore, the strategy depicted in figure 2 was
15 followed. Briefly, the PCR product was restricted with *Xba*I/*Pst*I and *Pst*I/*Bam*HI (figure 1; *Bam*HI site was introduced with GTFpr4). The resulting fragments (1503 bp and 2696 bp, respectively) were cloned separately in pBluescriptIIISK⁺ yielding pBXP1500 and pBPB2700. Ligation of the 2700 bp *Pst*I/*Sa*II fragment isolated from pBPB2700 in pBXP1500, digested with *Pst*I and *Sa*II, yielded pBGTF (7146 bp) in *E. coli* DH5 α .
20 Plasmid DNA of *E. coli* was isolated using the alkaline lysis method of Birnboim and Doly (1979) Nucleic. Acid Res. 7, 1513-1523 or with a Qiagen plamid kit following the instructions of the supplier. Cells of *E. coli* DH5 α with pBGTF were harvested by centrifugation after 16h of growth. The pellet was washed with 50 mM sodium acetate buffer pH 5.5 containing 1 mM CaCl₂ and 1% (v/v) Tween-80 and the suspension was
25 centrifuged again. Pelleted cells were resuspended in 50 mM sodium acetate buffer pH 5.5 containing 1 mM CaCl₂, 1% (v/v) Tween-80 and 7.2 mM β -mercaptoethanol. Cells were broken by sonication. Cell debris and intact cells were removed by centrifugation for 15 min at 4°C at 14,000 rpm in an Eppendorf centrifuge and the resulting cell free extract was used in the enzyme assays.

30 The glucosyltransferase activity was determined at 37°C by monitoring the release of fructose from sucrose or by measuring the amount of glucan produced using *E. coli* cell free extracts or *Lactobacillus reuteri* culture supernatant in reaction buffer (50 mM sodium acetate, 1 mM CaCl₂, 1% (v/v) Tween-80, 10 g/l sucrose, pH 8). Sucrose, glucose and fructose were determined using commercially available kits. For determination of the

molecular weight of the glucosyltransferase produced by *E. coli* or *L. reuteri*, SDS-PAGE was performed according to Laemmli (1970) Nature 227, 680-685. SDS-PAGE gels were stained using the PAS activity staining. Glucans were collected by precipitation with ethanol. ¹H-NMR spectroscopy (figure 6) and methylation analysis (table 1) were performed as described by van Geel-Schutten *et al.* (1999) Appl. Environ. Microbiol. 65, 3008-3014. The molecular weights of the glucans were determined by high performance size exclusion chromatography coupled on-line with a multi angle laser light scattering and a differential refractive index detector. After the first nucleotide sequencing of the obtained DNA two putative start codons leading to either a protein encoded by 3834 nucleotides (starting at nucleotide position 1670 of SEQ ID No. 1) or a protein encoded by 3753 nucleotides (starting at nucleotide position 1751 of SEQ ID No. 1) were identified. Both putative start codons were preceded by a putative ribosome binding site, GCAGG (located 4 base pairs upstream of nucleotide position 1670 of SEQ ID No. 1) or AGAAG (located 14 base pairs upstream of nucleotide position 1751 of SEQ ID No. 1), respectively. Depending on the potential start codon used, one of these glucosyltransferases comprised 1278 amino acids (starting at amino acid positions 504 of SEQ ID No. 2) (3834 nucleotides) and the other comprised 1251 amino acids (starting at amino acid position 531 of SEQ ID No. 2) (3753 nucleotides). The molecular weight (MW) deduced of the amino acid sequence of these glucosyltransferases was 143 and 140 kDa, respectively. The isoelectric point deduced of the amino acid sequence of these glucosyltransferases was 4.73 (for the higher MW protein) and 4.71 (for the lower MW protein), at pH 7, respectively. Surprisingly, the molecular weight of the purified protein from *Lactobacillus reuteri* indicated by SDS-PAGE was not approximately 140 kDa, but about 190 kDa. After repeating the nucleotide sequencing, it appeared that the above mentioned nucleotide forms did not represent the complete nucleotide sequence of the glucosyltransferase according to the invention, but were merely a part of the complete nucleotide sequence encoding the protein of the invention. The complete nucleotide sequence of the novel glucosyltransferase is represented in SEQ ID No. 1 and the amino acid sequence of said glucosyltransferase is shown in SEQ ID No. 2. All experiments were performed with both the complete nucleotide or amino acid sequence of the protein (SEQ ID No. 1 and 2, respectively) and the partial nucleotide or amino acid sequence mentioned above. The results of the experiments performed with the complete or partial amino acid sequence and the complete or partial nucleotide sequence mentioned above were identical indicating that the part of the glucosyltransferase represented by said partial nucleotide

and amino acid sequences is essential for the functionality of the glucosyltransferase according to the invention.

Table 1: Methylation analysis of the glucans produced by *Lactobacillus reuteri* strains and
5 *E. coli* GTFA.

Type of glucosyl units	<i>Lactobacillus reuteri</i> strain 121	<i>Lactobacillus reuteri</i> strain 35-5	<i>E. coli</i> GTFA
Glc _p -(1→	24%	25%	21%
→4)-Glc _p -(1→	42%	43%	44%
→6)-Glc _p -(1→	22%	21%	24%
→4,6)-Glc _p -(1→	12%	11%	11%

Example 2: Adhesion experiments with *Lactobacillus* strains.

The adhesion of *Lactobacillus reuteri* strains to Caco-2 cell lines was determined as described below. Firstly, a bacterial suspension was prepared as follows. *L. reuteri*
10 strains LB 121, 35-5 and *L. rhamnosus* LGG (a well known probiotic strain with good adhering properties) were cultured in MRS broth supplemented with 5 µl/ml of methyl-1,2-[³H]-thymidine at 37°C for 18-20 h before the adhesion assays. The cultures were harvested by centrifugation, washed with phosphate buffered saline (PBS) and resuspended in PBS or PBS supplemented with 30 g/l sucrose (see Table 2) to a final
15 density of about 2 x 10⁹ cfu/ml. Prior to the adhesion assay, the cell suspensions in PBS with 30 g/l sucrose were incubated for 1 hour at 37°C, whereas the cell suspensions in PBS were kept on ice for 1 hour. After incubation at 37°C, the suspensions in PBS with sucrose were centrifuged and the cells were washed with and resuspended in PBS to a final density of about 2 x 10⁹ cfu/ml.

20 Caco-2 cells were cultured as follows. Subcultures of Caco-2 cells (ATCC, code HTB 37, human colon adenocarcinoma), stored as frozen stock cultures in liquid nitrogen were used for the adhesion tests. The Caco-2 cells were grown in culture medium consisting of Dulbecco's modified Eagle medium (DMEM), supplemented with heat-inactivated foetal calf serum (10% v/v), non-essential amino acids (1% v/v), L-glutamine
25 (2mM) and gentamicin (50 µg/ml). About 2,000,000 cells were seeded in 75cm² tissue culture flasks containing culture medium and cultured in a humidified incubator at 37°C in air containing 5% CO₂. Near confluent Caco-2 cell cultures were harvested by

trypsinisation and resuspended in culture medium. The number of cells was established using a Bürker-Türk counting chamber.

Table 2: Incubation of the different *Lactobacillus* strains prior to the adhesion assays.

<i>Lactobacillus</i> strain	Extra incubation	Polysaccharide produced	Group
<i>reuteri</i> 121	PBS sucrose, 37°C, 1 hr	glucan and fructan	As
<i>reuteri</i> 35-5	PBS sucrose, 37°C, 1 hr	glucan	Bs
<i>reuteri</i> K24	PBS sucrose, 37°C, 1 hr	none	Cs
<i>reuteri</i> 121	PBS on ice	none	D
<i>reuteri</i> DSM20016*	PBS on ice	none	E
<i>rhamnosus</i> GG	PBS on ice	none	F

5 * Type strain of *L. reuteri*

For the following experiments a Caco-2 monolayer transport system was used. Caco-2 cells cultured in a two-compartment transport system are commonly used to study the intestinal, epithelial permeability. In this system the Caco-2 cell differentiates into polarised columnar cells after reaching confluency. The Caco-2 system has been shown to simulate the passive and active transcellular transport of electrolytes, sugars, amino acids and lipophilic compounds (K.M. Hillgren, A. Kato, R.T. Brochardt, Medical Research Reviews 15: 83-109, 1995; W.J. Dulfer, J.P. Groten, H.A.J. Govers, J. Lipid Res. 37: 950-961, 1996; E. Duizer, A.H. Penninks, W.H. Stenhuis and J.P. Groten, J. Contr. Rel. 49: 39- 49, 1997). Also, a clear correlation between the *in vivo* absorption and the permeability across the monolayers of Caco-2 cells has been reported (P. Artursson and J. Karlsson, Biochem. Biophys. Res. Commun. 75: 880-885, 1991). For the present transport studies, Caco-2 cells were seeded on semi-permeable filter inserts (12 wells Transwell plates, Costar) at ca. 100,000 cells per filter (growth area $\pm 1 \text{ cm}^2$ containing 2.5 ml culture medium). The cells on the insert were cultured for 17 to 24 days at 37°C in a humidified incubator containing 5% CO₂ in air. During this culture period the cells have been subjected to an enterocyte-like differentiation. Gentamycin was eliminated from the culture medium two days prior to the adhesion assays.

The adhesion assay was performed as follows. PBS was used as exposure medium. 25 μl of a bacterial suspension ($2 \times 10^9 \text{ cfu/ml}$) were added to 0.5 ml medium. The apical side of the Caco-2 monolayers was incubated with the bacterial suspensions for 1 hour at 37°C. After incubation, remaining fluid was removed and the cells were washed three

times with 1 ml PBS. Subsequently, the Caco-2 monolayers were digested overnight with 1 ml 0.1M NaOH, 1% SDS. The lysate was mixed with 10 ml Hionic Fluor scintillation liquid and the radioactivity was measured by liquid scintillation counting using a LKB/-Wallac scintillation counter. As a control, the radioactivity of the bacterial suspensions was measured. For each test group, the percentage of bacteria attached to the monolayers was calculated. All adhesion tests were performed in quadruple. In Table 3 the results of the bacterial adhesion test to Caco-2 cell lines are given. From the results can be concluded that the glucans and the fructans contribute to the adherence of *L. reuteri* to Caco-2 cell lines. This could indicate that *L. reuteri* strains producing EPS possess improved probiotic characteristics or that *L. reuteri* and its polysaccharides could function as an excellent symbiotic.

Table 3: The results of the bacterial adhesion test to Caco-2 cell lines.

Group (see Table 1)	% of bacteria bound to the monolayer
As	6.5
Bs	5.7
Cs	1.8
D	2.3
E	0.9
F	1.3

15 Example 3: Oxidation of glucans.

For TEMPO-mediated oxidation, a glucan produced as described above (dry weight 1 g, 6.15 mmol) was resuspended in 100 ml water. Next, 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO; 1% by weight compared to the polysaccharide (0.01 g, 0.065 mmol)) was added and resuspended in 20 min. Sodium bromide (0.75 g, 7.3 mmol) was added and the suspension was cooled to 0°C. The reaction can also be performed without bromide. A solution of hypochlorite (6 ml, 15% solution, 12.6 mmol) was adjusted to pH 10.0 with 3M HCl and cooled down to 0°C. This solution was added to the suspension of the polysaccharide and TEMPO. The course of the reaction was followed by monitoring the consumption of sodium hydroxide solution, which is equivalent to the formation of uronic acid. After 30 min, the amount of 0.1M NaOH consumed corresponded to the formation of 96% uronic acid, with respect to the available units having a primary

hydroxyl group. Thereafter, the solution was poured out in 96% ethanol (comprising 70% of the volume of the solution) causing the product to precipitate. The white precipitate was centrifuged, resuspended in ethanol/water (70/30 v/v) and centrifuged again. Next, the precipitate was resuspended in 96% ethanol and centrifuged. The obtained product was
5 dried at reduced pressure. The uronic acid content was determined by means of the uronic acid assay according to Blumenkrantz and Abdoe-Hansen (Anal. Biochem., 54 (1973), 484). A calibration curve was generated using polygalacturonic acid (5, 10, 15 and 20 µg). With this calibration curve the uronic acid content in a sample of 20 µg of the product was determined. The obtained result was a content of 95% uronic acid with respect to available
10 6-hydroxymethyl groups, the with a yield of 95%.

For partial oxidation (about 6%), a glucan produced as described before (dry weight 7.5 g, 46.4 mmol) was resuspended in 50 ml water. Next, TEMPO (1% by weight compared to the polysaccharide (0.075 g, 0.5 mmol)) was added, resuspended in 20 min and cooled to 0°C. A solution of hypochlorite (2.8 ml, 15% solution, 5.9 mmol) was
15 adjusted to pH 9.0 with 3M HCl and cooled down to 0°C. This solution was added to the suspension of the polysaccharide and TEMPO. Within 5 min the mixture became a solid gel.

Description of the figures

SEQ ID No. 2: The deduced amino acid sequence of glucosyltransferase A
20 (GTFA) of *Lactobacillus reuteri*.

Figure 1: The strategy used for the isolation of the *gtfA* gene from *Lactobacillus reuteri* 121 chromosomal DNA.

Figure 2: The general principle of the construction of the recombinant plasmid with the *gtfA* gene. A PCR product containing the *gtfA* gene was digested with *Xba*I and
25 *Pst*I and with *Pst*I and *Bam*HI. The *Xba*I/*Pst*I (depicted in white) was ligated into the multiple cloning site of pBluescriptIISK⁺ in the same direction relatively to the *lacZ* promotor, resulting in pBXP1500. The *Pst*I/*Bam*HI part (depicted in grey) was ligated into the multiple cloning site of pBluescriptIISK⁺ in the opposite direction relatively to the *lacZ* promotor, resulting in pBPB2700. pBXP1500 was used as a vector for subcloning the
30 C-terminal part of the *gtfA*. pBPB2700 was digested with *Pst*I and *Sal*I and ligated into pBXP1500, also digested with *Pst*I and *Sal*I. The resulting plasmid, pBGTF, contained the entire *gtfA* in the same direction relative to the *lacZ* promotor. The ⌘ sign indicates the restriction.

Figure 3: The nucleotide and deduced amino acid sequence of *gtfA* of *Lactobacillus reuteri*. The start codon is shown in bold. The putative ribosomal binding site (RBS) is also shown in bold. The nucleotides in italic and underlined upstream of the start codon indicate the -10 region (Pribnow box) and the -35 region. The inverted repeats (transcription termination) after the stop codon are underlined. The * sign indicates the stop codon.

Figure 4: Alignment of catalytic cores of alternansucrase (ASR) of *Leuconostoc mesenteroides* strain NRRL B-1355 dextranase (DSRS) of *Leuconostoc mesenteroides* strain NRRL B-512F, glucosyltransferase-D (GTFD) of *Streptococcus mutans* GS5, glucosyltransferase-A of *Lactobacillus reuteri* and amylosucrase (AS) of *Neisseria polysaccharea*. * indicates identical or conserved residues in all sequences; --, gap in the sequence; AA amino acids which are conserved in all other glucosyltransferases but not in GTFA; ↓, putative catalytic residues; •, putative calcium binding sites; ♦, putative residues stabilising the transition state; ∇, residues possibly playing a role in binding of acceptor molecules and in the transfer of the glucosyl residue; ◇, putative chloride binding sites; -Ex-, localisation of β-strands; -Hx-, localisation of α-helices according to Mac Gregor *et al.* (1996) FEBS Lett. 378, 262-266. The numbering of the amino acids of the glucosyltransferase-A of *Lactobacillus reuteri* corresponds to the positions of these amino acids in the amino acid sequence 531-1781 of amino acid sequence SEQ ID No. 2, when the amino acid sequence 531-1781 is renumbered 1-1251.

Figure 5: Dendrogram of glucansucrases of lactic acid bacteria. The horizontal distances are a measure for the difference at the amino acid sequence level. 10% difference is indicated by the upper bar. Bootstrap values (in percentages) are given at the root of each branch.

Figure 6: 500-MHz ¹H-NMR spectra of the glucan produced by *Lactobacillus reuteri* GTFA (A) and by *E. coli* GTFA (B), recorded in D₂O at 80°C.

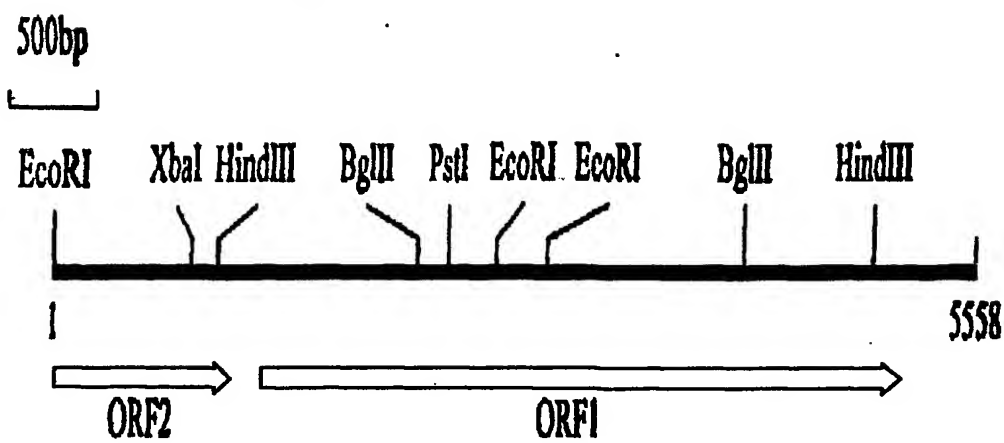
Claims

1. A protein having glucosyltransferase activity comprising an amino acid sequence, exhibiting at least 50% amino acid identity, as determined by the BLAST algorithm, with the amino acid sequence 531-1781 of SEQ ID No. 2 or a part thereof having at least 15 contiguous amino acids which are identical to the corresponding part of the amino acid sequence 531-1781 of SEQ ID No. 2.
2. A protein according to claim 1 comprising an amino acid sequence, exhibiting at least 60%, preferably at least 70%, amino acid identity with the amino acid sequence 531-1781 of SEQ ID No. 2.
3. A protein according to claim 1 or 2, exhibiting at least 50% amino acid identity, as determined by the BLAST algorithm, with the amino acid sequence of SEQ ID No. 2 or a part thereof having at least 15 contiguous amino acids which are identical to the corresponding part of the amino acid sequence of SEQ ID No. 2.
4. A protein according to claim 3, exhibiting at least 60%, preferably at least 70%, amino acid identity with the amino acid sequence of SEQ ID No. 2.
5. A protein according to any one of claims 1-4, comprising an amino acid sequence of at least 200 amino acids exhibiting at least 55%, preferably at least 65%, amino acid identity with the corresponding part of the amino acid sequence 972-1514 of SEQ ID No. 2.
6. A protein according to any one of claims 1-5, comprising an amino acid sequence of at least 100 amino acids exhibiting at least 50%, preferably at least 60%, amino acid identity with the corresponding part of the amino acid sequence 1515-1781 of SEQ ID No. 2.
7. A protein according to any one of claims 1-6, comprising at least one of the amino acids Pro-1026, Ile-1029, Met-1034, Asn-1035, Ser-1136, Ala-1143, Ile-1170, Leu-1223, Ala-1413, Val-1418, Ala-1428, Leu-1442 of the amino acid sequence of SEQ ID No. 2.
8. A protein according to any one of the preceding claims which, in the presence of sucrose, produces a glucan having 38-48% 4-linked anhydroglucose units, 17-28% 6-linked anhydroglucose units, and 7-20% 4,6-linked anhydroglucose units.

9. A protein according to any one of the preceding claims which is a recombinant protein.
10. A nucleotide sequence encoding a protein according to any one of the preceding claims.
11. A nucleic acid construct comprising the nucleic acid sequence of claim 10, operationally linked to an expression-regulating nucleic acid sequence.
12. A recombinant host cell containing one or more copies of the nucleic acid construct according to claim 11.
13. A process of producing a protein of interest, such as a glucosyltransferase, comprising culturing a host cell according to claim 12 in a culture medium, and recovering the protein from the culture medium or the cell free extract.
14. A process of producing an oligosaccharide or polysaccharide of interest, using a protein according to any one of claims 1-9, or a host cell according to claim 12.
15. A chemically modified glucan, which is obtained by 2,3-oxidation, 6-oxidation, phosphorylation, acylation, hydroxyalkylation, carboxymethylation, aminoalkylation of one or more anhydroglucose units of a glucan comprising at least 20, up to about 100,000 α -anhydroglucose units, 38-48% of which are 4-linked anhydroglucose units, 17-28% are 6-linked anhydroglucose units, and 7-20% are 4,6-linked anhydroglucose units.
16. Use of a *Lactobacillus* strain capable of producing a glucan comprising at least 20, up to about 100,000 α anhydroglucose units, 38-48% of which are 4-linked anhydroglucose units, 17-28% are 6-linked anhydroglucose units, and 7-20% are 4,6-linked anhydroglucose units with sucrose as a substrate and optionally a fructan as a probiotic or symbiotic.

1/17

Fig 1



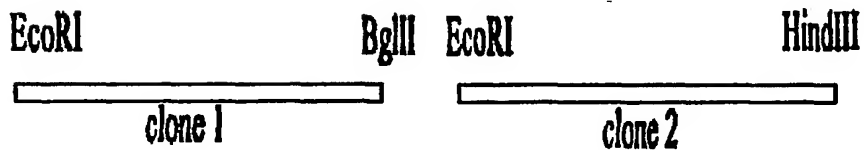
a)



b)



c)



d)



2/17

Fig 2

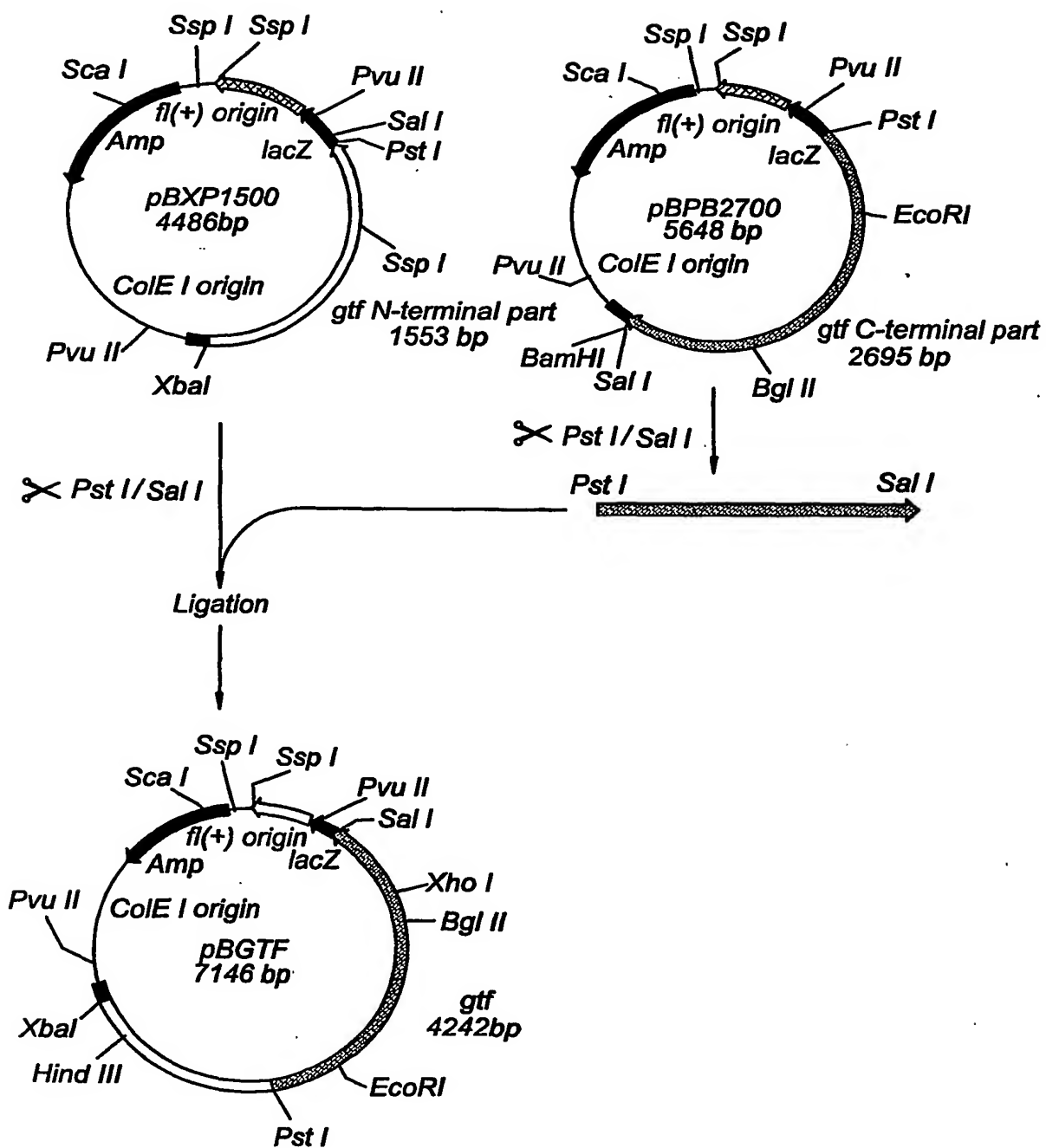


Fig.3.1

3/17

```

1  ATACATATTTTGGGCTTCTTTTTTTGTTTAAAACTGTAAATTTGAATTTTATTTGAAAAA
1  Y I F W A S F F V * N C K F E F Y L K K
      -35                                -10
61  ATTTTGGCTAGAATTTGAAAATTCCCTTTGAAAAAATAAAACATCATAGTATTATAATAC
21  F W L E F E N S L * K N K T S * Y Y N T
      RBS                                start
121 CGATAATCAAATTGTTTATTTTGATATGAAGGAGATTAAATGGAAATAAAGAAACATTT
41  D N Q I V Y F D M K E I K M E I K K H F

181 TAAGTTGTACAAAAGCGGCAAACAATGGGTAACAGCAGCAGTGGCTACCGTTGCCGTTTC
61  K L Y K S G K Q W V T A A V A T V A V S

241 AACTGCGCTTCTTTACGGAGGAGTTGCGCATGCTGACCAACAAGTTCAGCAAGCTTCCAC
81  T A L L Y G G V A H A D Q Q V Q Q A S T

301 GACTCAAGACCAAACTTCTACCGTAAATAATGATACTGATAAAACAGTAGCTTTAGATAC
101 T Q D Q T S T V N N D T D K T V A L D T

361 TAATACTGACCAGTCAGCTCAAACAACCTGATAAAAAACAAGTAGTATCAAATACTAACCA
121 N T D Q S A Q T T D K K Q V V S N T N Q

421 AAGCAAACTGATGACACTTCAACAGCTGATAAGAATTCTACTTCTACACCTGTTTCTGT
141 S K T D D T S T A D K N S T S T P V S V

481 TTTGCCATCTAATAATACTGAAAAACAAGCTAAAAATTATAATGAGCAAGACAAAGGAAA
161 L P S N N T E K Q A K N Y N E Q D K G N

541 CTATGGGAATATTGATACTGCTTACTTTAGCAATAATCAATTGCATGTTTCAGGATGGAA
181 Y G N I D T A Y F S N N Q L H V S G W N

601 TGCAACGAACGCATCTCAAGGAACAAACAGTCGACAAATCATTGTGCGTGATATCACAAC
201 A T N A S Q G T N S R Q I I V R D I T T

661 CAATAATGAATTAGGTCGCACTGATGTAACAAACAATGTTGCACGCCCCAGACGTTAAGAA
221 N N E L G R T D V T N N V A R P D V K N

```

4/17

Fig.3.2

721 TGTCATAATGTTTATAACGCTGATAATTCTGGATTGATGTTAATGTCAATATTGACTT
241 V H N V Y N A D N S G F D V N V N I D F

781 TAGCAAGATGAAAGATTATCGGGATTCAATTGAAATTGTTAGTCGATACAGTGGAAACGG
261 S K M K D Y R D S I E I V S R Y S G N G

841 TAAATCTGTTGACTGGTGGTCCCAACCGATCACTTTTGACAAAAACAACATGCTTATCT
281 K S V D W W S Q P I T F D K N N Y A Y L

901 TGATACATTGGAAGTGAAAAATGGCGAATTACATGCAACCGGATGGAATGCTACTAATAG
301 D T F E V K N G E L H A T G W N A T N S

961 TCGGATTAACATAATCACCATTTTGTGATTTTGTGTTGATCAAACGAATGGTAAAGAAGT
321 A I N Y N H H F V I L F D Q T N G K E V

1021 AGCACGACAAGAAGTTCGTGAAGGTCAATCACGCCCGAGATGTTGCTAAGGTATATCCACA
341 A R Q E V R E G Q S R P D V A K V Y P Q

1081 AGTAGTTGGTGCTGCCAACTCAGGCTTTAATGTGACATTTAATATCAGTGATTAGATTA
361 V V G A A N S G F N V T F N I S D L D Y

1141 TACTCACCAGTACCAAGTTCTTAGTCGTTACAGCAATTCTGATAATGGCGAAGGTGATAA
381 T H Q Y Q V L S R Y S N S D N G E G D N

1201 CGTTACCTACTGGTTTAATCCACAATCCATTGCTCCTGCTAATCAAAGTAACCAGGGTTA
401 V T Y W F N P Q S I A P A N Q S N Q G Y

1261 TCTAGACTCATTGATATTAGTAAAAATGGTGAAGTAACAGTAACAGTGGATGGAACGCTAC
421 L D S F D I S K N G E V T V T G W N A T

1321 TGAATTGTCAGAATTACAAAACAACCATTATGTGATTCTATTTGATCAGACAGCAGGCAA
441 D L S E L Q N N H Y V I L F D Q T A G K

Fig.3.3

5/17

1381 ACAAGTTGCATCTGCTAAAGCTGATTTAATTTACGTCAGATGTTGCTAAAGCTTATCC
461 Q V A S A K A D L I S R P D V A K A Y P

1441 AACAGTAAAAACAGCTACAAATCTGGCTTCAAGGTAACATTTAAGGTTAATAACTTACA
481 T V K T A T N S G F K V T F K V N N L Q

1501 ACCGGGTCACCAATACAGCGTTGTAAGTCGTTTCTCTGCCGATGAAAATGGTAATGGTAA
501 P G H Q Y S V V S R F S A D E N G N G N

1561 TGATAAGCGCCATACAGATTACTGGTTTAGTCCAGTAATATTAAACCAGACTGCTTCAAA
521 D K R H T D Y W F S P V I L N Q T A S N

1621 CATTGATACTATTACAATGACATCTAATGGTTTACATATTGCAGGTTGGATGGCAAGTGA
541 I D T I T M T S N G L H I A G W M A S D

1681 TAACTCAATTAATGAAACAACTCCATACGCTATTATCCTCAATAATGGAAAAGAAGTTAC
561 N S I N E T T P Y A I I L N N G K E V T

1741 TCGTCAAAAGATGAGCTTAACCGCCCGTCCAGATGTAGCAGCAGTATATCCTTCACTTTA
581 R Q K M S L T A R P D V A A V Y P S L Y

1801 TAATAGTGCTGTTAGTGGTTTTGACACTACTATTAAATTGACTAATGATCAATATCAAGC
601 N S A V S G F D T T I K L T N D Q Y Q A

1861 GCTTAATGGCCAATTACAAGTATTGTTACGTTTTTCTAAAGCTGCTGATGGTAATCCAAG
621 L N G Q L Q V L L R F S K A A D G N P S

1921 TGGTGATAATACTGTAACTGATCAATTTAGTAAAAATTATGCAACTACTGGTGGAACTT
641 G D N T V T D Q F S K N Y A T T G G N F

1981 TGACTATGTAAAAGTAAATGGTAATCAAGTTGAATTTAGTGGTTGGCACGCAACTAACCA
661 D Y V K V N G N Q V E F S G W H A T N Q

6/17

Fig.3.4

2041 ATCAAATGATAAAGATTCAACAATGGATTATTGTTTTAGTTAATGGTAAGGAAGTAAAGCG
681 S N D K D S Q W I I V L V N G K E V K R

2101 TCAATTAGTTAATGATACTAAAGAGGGAGCTGCTGGCTTCAACCGAAACGATGTCTACAA
701 Q L V N D T K E G A A G F N R N D V Y K

2161 AGTAAATCCAGCTATTGAAAACAGTTCTATGTCTGGATTCCAAGGCATTATTACTTTACC
721 V N P A I E N S S M S G F Q G I I T L P

2221 TGTGACAGTTAAAAACGAAAATGTCCAACCTTGTTTCATCGGTTTAGTAACGATGTGAAGAC
741 V T V K N E N V Q L V H R F S N D V K T

2281 TGGTGAAGGTAACCTATGTTGATTTCTGGTCAGAGCTAATGCCTGTTAAGGATAGCTTCCA
761 G E G N Y V D F W S E L M P V K D S F Q

2341 AAAGGGGAATGGCCCACTTAAGCAATTTGGCTTACAACTATTAACGGTCAACAATATTA
781 K G N G P L K Q F G L Q T I N G Q Q Y Y

2401 TATTGACCCAACAACCTGGTCAACCACGTAAGAATTTCTTATTACAAAGTGGAAATAATTG
801 I D P T T G Q P R K N F L L Q S G N N W

2461 GATTTACTTTGATAGTGATACTGGTGTGGGTACTAATGCACTTGAATTACAATTTGCAAA
821 I Y F D S D T G V G T N A L E L Q F A K

2521 GGGAACTGTTTCATCTAATGAACAATACCGTAACGGTAATGCAGCTTACAGTTATGATGA
841 G T V S S N E Q Y R N G N A A Y S Y D D

2581 CAAGAGTATCGAAAATGTAAATGGTTACTTAACAGCAGATACATGGTACCGTCCAAAACA
861 K S I E N V N G Y L T A D T W Y R P K Q

2641 GATCTTAAAGGATGGAACCTACCTGGACTGACTCAAAAGAAACAGATATGCGACCAATCTT
881 I L K D G T T W T D S K E T D M R P I L

2701 GATGGTATGGTGGCCTAATACTCTTACCCAAGCATACTACCTTAATTACATGAAACAACA

7/17

Fig.3.5

901 M V W W P N T L T Q A Y Y L N Y M K Q H
2761 TGGTAATTTATTACCATCTGCTTTACCATTCCTTTAATGCGGATGCTGATCCTGCAGAATT
921 G N L L P S A L P F F N A D A D P A E L
2821 AAATCATTATTCCGAAATTGTGCAACAAATATTGAAAAACGAATTAGTGAAACCGGAAA
941 N H Y S E I V Q Q N I E K R I S E T G N
2881 TACTGATTGGTTACGTACTTTAATGCACGATTTTGTACTAACAATCCGATGTGGAATAA
961 T D W L R T L M H D F V T N N P M W N K
2941 GGATAGTGAAAATGTTAACTTTAGTGGTATTCAATTCCAAGGCGGATTCTTAAAGTATGA
981 D S E N V N F S G I Q F Q G G F L K Y E
3001 AAATCAGATTTAACGCCTTATGCTAACTCTGATTATCGCTTACTTGGTCGGATGCCAAT
1001 N S D L T P Y A N S D Y R L L G R M P I
3061 CAATATTAAGGATCAAACATATCGGGGACAAGAATTCCTACTTGCTAACGATATTGATAA
1021 N I K D Q T Y R G Q E F L L A N D I D N
3121 CTCTAATCCTGTTGTTCAAGCAGAACAATTAACTGGTTATACTATCTCTTGAACCTTTGG
1041 S N P V V Q A E Q L N W L Y Y L L N F G
3181 AACGATCACAGCTAATAATGATCAAGCTAATTTTGATTCTGTACGGGTAGATGCACCGGA
1061 T I T A N N D Q A N F D S V R V D A P D
3241 TAATATTGATGCCGATCTTATGAATATCGCTCAGGACTACTTTAATGCTGCATATGGTAT
1081 N I D A D L M N I A Q D Y F N A A Y G M
3301 GGAATCAGATGCTGTCTCAAATAAGCATATTAATATTCTTGAAGACTGGAATCATGCTGA
1101 D S D A V S N K H I N I L E D W N H A D
3361 TCCGGAATACTTTAATAAGATCGGAAATCCACAATTGACAATGGATGATACTATTAAGAA
1121 P E Y F N K I G N P Q L T M D D T I K N

SUBSTITUTE SHEET (RULE 26)

8/17

Fig.3.6

3421 TTCCCTGAATCATGGGCTTTCAGATGCAACTAATCGTTGGGGATTAGATGCAATTGTTCA
1141 S L N H G L S D A T N R W G L D A I V H

3481 TCAGTCATTAGCTGATCGTGAAAATAATTCCACGGAAAATGTTGTAATTCCTAATTACAG
1161 Q S L A D R E N N S T E N V V I P N Y S

3541 TTTCGTTCTGGGCTCACGATAATAATTCTCAAGATCAAATTCAAAATGCTATTCGTGATGT
1181 F V R A H D N N S Q D Q I Q N A I R D V

3601 AACAGGCAAAGATTACCATACTTTCACCTTTTGAAGATGAGCAAAGGGTATTGATGCGTA
1201 T G K D Y H T F T F E D E Q K G I D A Y

3661 CATTCAAGATCAAATTCACAGTGAAGAAATATAACCTTTATAATATTCGGGCTTCATA
1221 I Q D Q N S T V K K Y N L Y N I P A S Y

3721 CGCAATTCTTTTAACTAACAAGGATACAATTCACGTGTATACTATGGTGACTTGTATAC
1241 A I L L T N K D T I P R V Y Y G D L Y T

3781 TGATGGTGGCCAATACATGGAACATCAAACACGTTACTATGATACTTTAACGAACCTGCT
1261 D G G Q Y M E H Q T R Y Y D T L T N L L

3841 TAAATCACGAGTTAAGTATGTTGCCGGTGGCCAATCAATGCAAACAATGAGCGTTGGCGG
1281 K S R V K Y V A G G Q S M Q T M S V G G

3901 CAATAATAACATTTTAACTAGTGTTTCGTTATGGTAAAGGTGCGATGACAGCTACTGATAC
1301 N N N I L T S V R Y G K G A M T A T D T

3961 TGGTACTGATGAAACCAGAACACAAGGTATTGGGGTTGTTGTAAGTAATACGCCAAATCT
1321 G T D E T R T Q G I G V V V S N T P N L

4021 AAAGCTAGGTGTCAACGATAAAGTAGTTCTTCATATGGGAGCTGCGCACAAGAACCAACA
1341 K L G V N D K V V L H M G A A H K N Q Q

9/17

Fig.3.7

4081 ATATCGGGCAGCCGTGTTGACGACAACTGATGGAGTCATTAATTATACTTCTGATCAAGG
1361 Y R A A V L T T T D G V I N Y T S D Q G

4141 GGCACCGGTTGCAATGACTGACGAGAACGGTGATCTATACTTATCTAGTCATAACCTAGT
1381 A P V A M T D E N G D L Y L S S H N L V

4201 TGTTAATGGTAAAGAAGAAGCAGATACAGCTGTTCAAGGTTATGCTAACCCCTGATGTTTC
1401 V N G K E E A D T A V Q G Y A N P D V S

4261 AGGATATCTTGCTGTATGGGTACCAGTTGGAGCAAGTGATAACCAAGATGCTCGAACTGC
1421 G Y L A V W V P V G A S D N Q D A R T A

4321 TCCATCTACTGAAAAGAATAGTGGTAACTCTGCATACAGAACAAATGCTGCTTTTGATTC
1441 P S T E K N S G N S A Y R T N A A F D S

4381 AAATGTTATTTTTGAAGCCTTTTCTAACTTTGTCTATACACCAACAAAGGAAAGTGAACG
1461 N V I F E A F S N F V Y T P T K E S E R

4441 TGCTAATGTTGGAATTGCCCAAATGCTGATTTCTTTGCTTCATTAGGTTTTACTTCTTT
1481 A N V R I A Q N A D F F A S L G F T S F

4501 CGAGATGGCGCCACAATATAATTCAAGTAAAGATCGCACATTCCTAGATTCAACAATTGA
1501 E M A P Q Y N S S K D R T F L D S T I D

4561 TAACGGATATGCGTTTACTGATCGTTATGATCTTGAATGAGTGAGCCTAATAAGTACGG
1521 N G Y A F T D R Y D L G M S E P N K Y G

4621 AACAGATGAAGATCTACGTAATGCCATTCAAGCGCTCCATAAAGCTGGCTTACAAGTAAT
1541 T D E D L R N A I Q A L H K A G L Q V M

4681 GGCGGATTGGGTTTCCTGACCAAATCTATAACCTTCCTGGAAAAGAAGTTGCTACAGTCAC
1561 A D W V P D Q I Y N L P G K E V A T V T

4741 TCGAGTAGATGATCGTGGTAATGTATGGAAAGATGCTATCATTAAATAATAATCTGTATGT
1581 R V D D R G N V W K D A I I N N N L Y V

10/17

Fig.3.8

4801 TGTTAATACTATTGGTGGTGGCGAATACCAGAAGAAGTATGGTGGAGCATTCCTCGATAA
1601 V N T I G G G E Y Q K K Y G G A F L D K

4861 GTTACAAAACTTTATCCTGAAATCTTCACAAAGAAGCAAGTTTCAACTGGTGTGCTAT
1621 L Q K L Y P E I F T K K Q V S T G V A I

4921 TGATCCTTCACAAAAGATAACTGAATGGTCAGCAAAATACTTTAATGGAACAAACATTCT
1641 D P S Q K I T E W S A K Y F N G T N I L

4981 CCATCGTGGTTCTGGTTATGTACTAAAAGCTGATGGTGGTCAATACTACAACCTTAGGTAC
1661 H R G S G Y V L K A D G G Q Y Y N L G T

5041 TACTACAAAGCAATTCTTGCCAATTCAATTAAGTGGTGAAAAGAAACAAGGAAATGAAGG
1681 T T K Q F L P I Q L T G E K K Q G N E G

5101 CTTTGTTAAGGGTAATGATGGAAATTACTACTTCTATGACTTAGCAGGTAATATGGTTAA
1701 F V K G N D G N Y Y F Y D L A G N M V K

5161 GAATACCTTTATTGAAGATAGTGTTGGCAACTGGTACTTCTTTGACCAAGATGGTAAGAT
1721 N T F I E D S V G N W Y F F D Q D G K M

5221 GGTTGAAAATAAACATTTTCGTTGATGTTGATTCTTATGGTGAAAAGGTACTTACTTCTT
1741 V E N K H F V D V D S Y G E K G T Y F F

5281 CTTGAAGAATGGTGTATCATTCCTGCGGGGATTAGTGCAAACTGACAATGGTACTTATTA
1761 L K N G V S F R G G L V Q T D N G T Y Y

5341 CTTTGATAATTATGGAAAGATGGTACGTAATCAAACCTATTAATGCAGGTGCCATGATTTA
1781 F D N Y G K M V R N Q T I N A G A M I Y

5401 TACCTTAGATGAAAACGGTAAGCTTATAAAGGCTAGTTATAATTCAGATGCCGAATATCC
1801 T L D E N G K L I K A S Y N S D A E Y P

11/17

Fig.3.9

5461 AACTTCAACTGATGTTGGTAAGATGCTTGATCAAAATAAACTATAAATGGAAATAATTAG
1821 T S T D V G K M L D Q N K L * M E I I S

5521 CTGATTTCCGTTTCTTAGAATCGAAAGATTTAATAACTGGGGTTAAACGGCCCTACAAA
1841 * F P F L R I E R F N N W G * N G P T K

5581 ATCTGATATTGATATAGAGATATTATTTCTATATCAATATCAGATTTTTGCTTTTTATA
1861 S D I D I E I L F P I S I S D F C F L *

5641 AAATTGATTGTGACTAATAAGAATCCGGAAGATAACGTTGTTGTTATATCAGTGGATTTA
1881 N * L * L I R I R K I T L L L Y Q W I *

5701 AGCAACATGAATTAATTGAAGATGACGGCAATGATTAAAAGTCGGTCTGATGATTATTGA
1901 A T * I N * R * R Q * L K V G L M I I D

5761 TGTATTACTAGTATTTGGTTTTTATCATTATATTTTTACTGTTATTGGTGTCATATATT
1921 V L L V F G F Y H L Y F Y C Y W C H I F

5821 CCACAATAACAGTAAAGGTATATATGCTAGTTTATTTTTTAAAGTAATTATAATATTCTGA
1941 H N N S K G I Y A S L F F K * L * Y S D

5881 TTATAATTTGGAAATATTCGCTTTTAGCAAAAAGGTAGTAAACAGATCAGAATCGTCATT
1961 Y N L E I F A F S K K V V N R S E S S F

5941 CTGCTTTTCTACTACTAAAAGTCTGTTTTAAATTCTAAACTAAAATAGGCTAAACACTGA
1981 C F S T T K S L F * I L N * N R L N T D

6001 TGTTTATCATTATATTTTTTACTGTT
2001 V Y H L Y F Y C

12/17

Fig. 4.1

♦ ↓ ∇∇

GTFD LLANDIDNSNPVVQAEQLNWLHYLMNYGSIVANDPEANFDGVRVDAVDNVNADLLQIASD 480
 DSRS LLANDVDNSNVVVEAEQLNWLHYLMNFGTITANDADANFDGIRVDAVDNVADLLQIAAD 576
 ASR LLANDIDNSNPIVQAEQLNWLHYLMNFGSITGNNDNANFDGIRVDAVDNVADLLKIAGD 650
 GTFA LLANDIDNSNPVVQAEQLNWLHYLLNFGTITANNDQANFDSVRVDAPDNIDADLMNIAQD 509
 ***** . ***** ** . * * * * * ***** ** . *** . * * *
 AS 262QWDLN₂₆₆ .290IVRMDAVAFL₂₉₈
 -----H3----- --E4-----H4--

↓∇∇∇

GTFD YLKAHYGVDKSEKNAINHLSILEAWSNDNPQYNKDTKGAQLPIDNKLRLSLLYALTRPLE 540
 DSRS YFKLAYGVDQNDATANQHLSILEDWSHNDPLYVTDQGSNQLTMDDYVHTQLIWSLTK--S 634
 ASR YFKALYGTDKSDANANKHLSILEDWNGKDPQYVNQQGNAQLTMDYTVTSQFGNSLTHGAN 710
 GTFA YFNAAYGMD-SDAVSNKHINILEDWNHADPEYFNKIGNPQLTMDDTIK----NSLNHGLS 564
 * ** * . . * . *** * ** * ** . * . *..
 ---- -E5-- ---H5--- ---E6--

GTFD KDASNKNEIRSGLEPVITNSLN-----NRSAEGKNSERMANYIFIRA 582
 DSRS ---SD---IRGTMQRFVDYIMV-----DRSNDSTENEAI PNYSFVRA 660
 ASR N-RSN---MWYFLDTGYLLNGDLNKKIVDKNRPNSGTLVNRIANS GDTKVIPNYSFVRA 765
 GTFA D-ATN---RWGLDAIVHQS-----LADRENNSTENVVIPNYSFVRA 601
 * . ** * . **
 AS 396FVRS
 -----H6----- --E7--

13/17

Fig. 4.2



GTFD HDSEVQTVIAKIIKAQINPKTDGLTFTLDELKQAFKIYNEDM--RQAKKKYTQSNIPTAY 640
 DSRS HDSEVQTVIAQIVSDLYPDVENS LAPTTEQLAAAFKVYNEDE--KLADKKYTQYNMASAY 718
 ASR HDYDAQDP IRKAMIDHGI IKNMQDTFTFDQLAQGMEFYKQDENPSGFKKYNDYNLPSAY 825
 GTFA HDNNSQDQIQNAIRDVTGKD--YHTFTFEDEQKGIDAYIQDQ-N-STVKKYNLYNIPASY 657
 ** * * . . * . * * * . * . . *

AS HD₄₀₁

----H7-

GTFD ALMLSNKDSITRLYYGDMYSDDGQYMATKSPYYDAIDTLLKARIKYAAGGQDMKITYVEG 700
 DSRS AMLLTNKDTVPRVYYGDLYTDDGQYMATKSPYYDAINTLLKARVQYVAGGQ----- 769
 ASR AMLLTNKDTVPRVYYGDMYLEGGQYMEKGTIYNPVISALLKARIKYVSGGQTMATDSSGK 885
 GTFA AILLTNKDTIPRVYYGDLYTDGGQYMEHQTRYDYDTLTNLLKSRVKYVAGGQSMQTM SVG- 716
 * . . * . * . . * . * . * . * . * . * . * . * . * . * . * . *

AS ₄₈₈GLPRIYLGD₄₉₆

H7-

--E8-

-----H8-----

GTFD DKSHMDWDYTGVLTSVRYGTGANEATDQGSEATK----TQGMVITSNNPSLKLNQNDKV 756
 DSRS ---SMSVDSNDVLT SVRYGKDAMTASDTGTSETR----TEGIGVIVSNNAELQLEDGHTV 822
 ASR DL---KDGETDLLTSVREGKGIMTSDQTTTQDNSQDYKNQGIGVIVGNPNLKLNDKTI 942
 GTFA -----GNNNILT SVRYGKGAMTATDTGTDETR----TQGIGVVVSNTPNLKLGVNDKV 765
 . . * . * . * * . * . * . * . *

GTFD IVNMGAAHKNQ EYRPLLLTTKDGLTSYTSDA AAKSLYRKTN D-----K-GELVFD 805
 DSRS TLHMGA A HKNQAYRALLSTTADGLAYYDTDENAPVAYTDAN-----GD LIFT 869
 ASR TLHMGKA HKNQLYRALVLSNDSGIDVYDSDDKAPT LR TN DNGDLIFHKTNTFVKQDGTII 1002
 GTFA VLHMGA A HKNQYRAAVLT TTDGVINYTS DQ GAPVAMTDENG DLYLSSHNLVVNGK-EEA 824
 . . * . * . * . * . . . * . * . * . *

14/17

Fig. 4.3

GTFD ASDIQGYLNPQVSGYLAVWVPVGASDNQDVRVAASNKANATG-QVYESSSALDSQLIYEG 864
 DSRS NESIYGVQNPQVSGYLAVWVPVGAQQDQDARTASDTTNTSD-KVFHSNAALDSQVIYEG 928
 ASR NYEMKGSNLALISGYLGVWVPVGASDSQDARTVATESSSSNDGSVFHSNAALDSNVIYEG 1062
 GTFA DTAVQGYANPDVSGYLAVWVPVGASDNQDARTAPSTEKNSGN-SAYRTNAAFDSDNVIFE_A 883

. * * .**** * * * * . . . * * * . *

-E1-

GTFD FSNFQDFVTKDSDYTNKKIAQNVQLFKSWGVTSEMAPQYVSS_{EDG}-----SFLDSIIQN 919
 DSRS FSNFQAFATDSSEYTNVIAQNA_{DQ}FKQWGVTSE_QLAPQYRSSTD_T-----SFLDSIIQN 983
 ASR FSNFQAMPTSPEQSTNVVIATKANLFKELGITSFELAPQYRSSGDTNYGGMSFLDSFLNN 1122
 GTFA FSNFVYTPTKESERANVRIAQNA_{DFF}ASLGFTSFEMAPQYNSSKDR-----TFLDSTIDN 938

**** * . * * * * * * * * * * * * * * * *

AS

₁₃₄GLTYLHLM_{P142}

---H1---

--E2-

GTFD GYAFEDRYDLAMSKN--N----KYGSQQDMINAVKALHKSGIQVIADWVPDQ 965
 DSRS GYAFTDRYDLGYGTP--T----KYGTADQLRDAIKALHASGIQAIADWVPDQ 1029
 ASR GYAFTDRYDLGFNKADGNPNPTKYGT_{DQ}DLRNAIEALHKNGMQAIADWVPDQ 1174
 GTFA GYAFTDRYDLGMSEP--N----KYGTDEDLRNAIQALHKAGLQVMADWVPDQ 984

**** * * * * * * * * * * * * * * * *

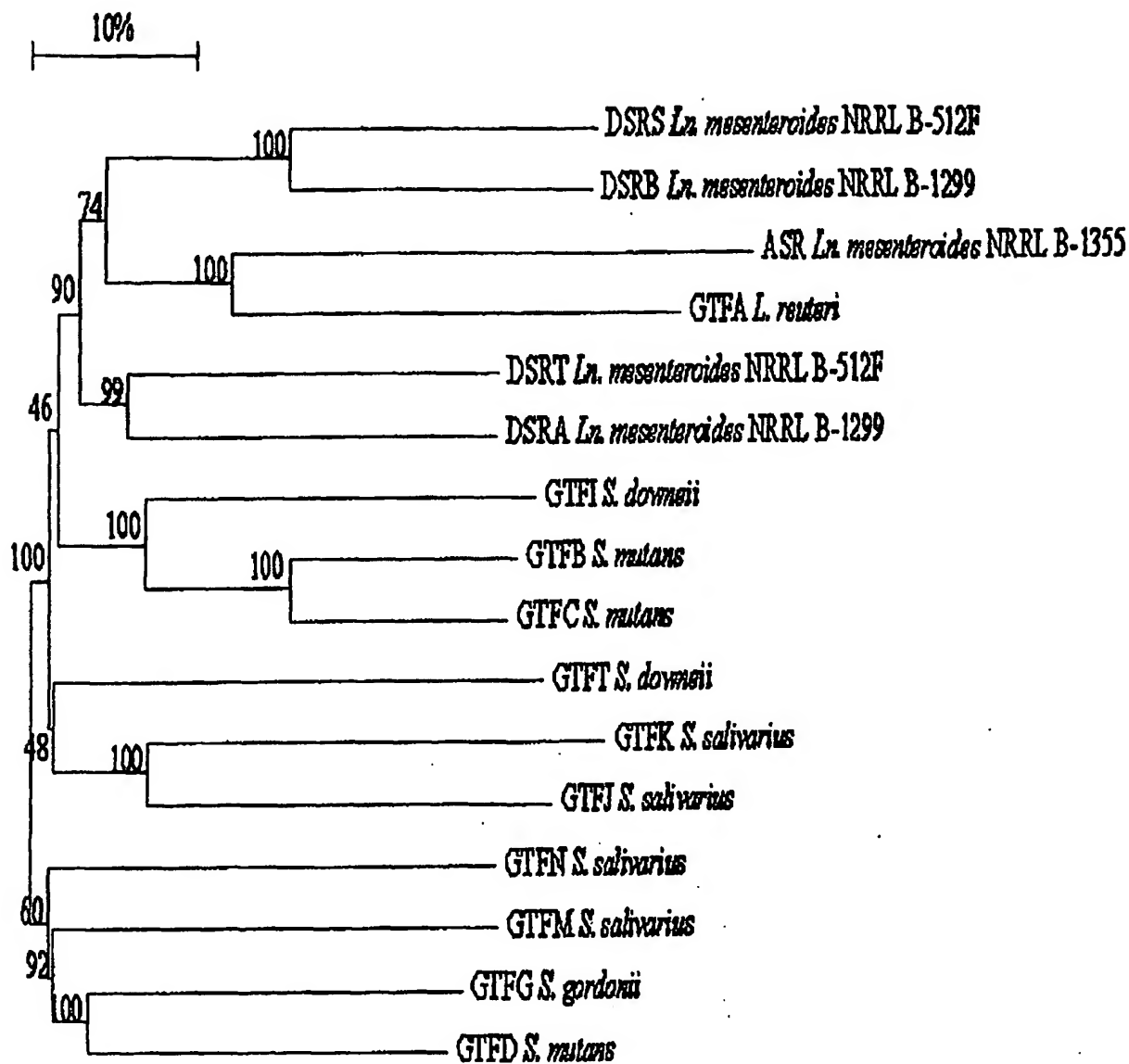
AS

₁₉₀DFITNH₁₉₅

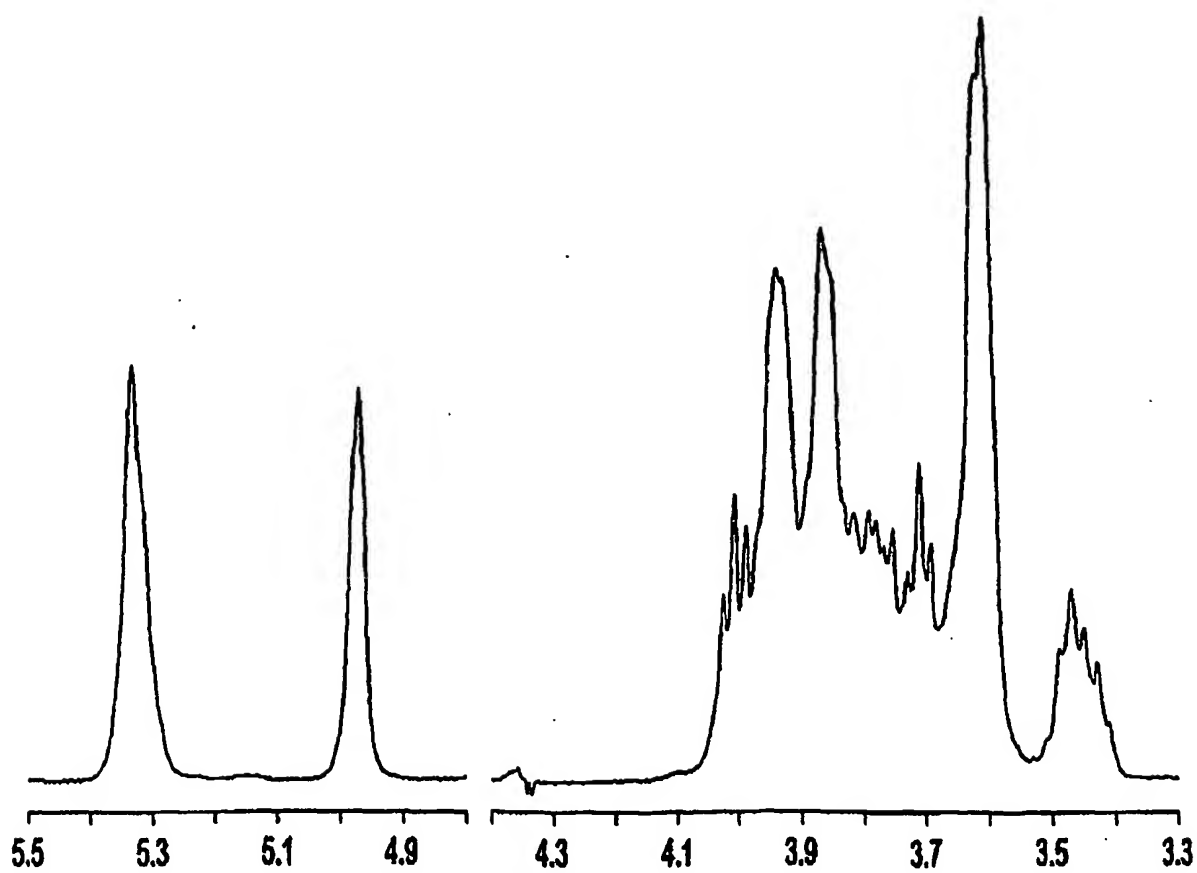
-----H2----- ---E3---

15/17

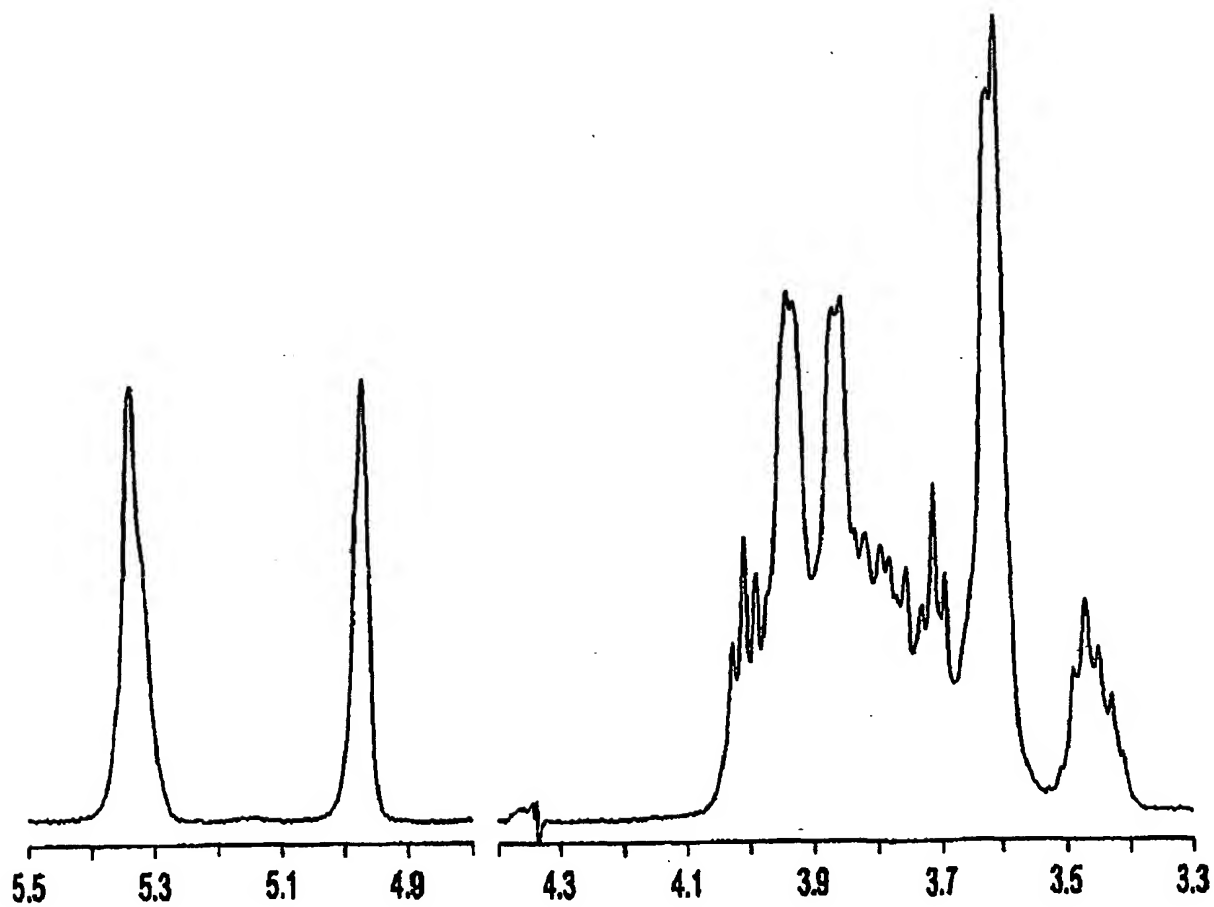
Fig. 5



16/17

Fig. 6a

17/17

Fig. 6b

SEQUENCE LISTING

<110> Nederlandse Organisatie voor TNO

<120> Novel glucosyltransferase

<130> Novel glucosyltransferase

<140>

<141>

<150> 00201871.1

<151> 2000-05-25

<160> 9

<170> PatentIn Ver. 2.1

<210> 1

<211> 6026

<212> DNA

<213> Lactobacillus reuteri

<220>

<221> RBS

<222> (148)..(155)

<220>

<221> -10_signal

<222> (113)..(118)

<220>

<221> -35_signal

<222> (75)..(80)

<220>

<221> CDS

<222> (161)..(5506)

<400> 1

atacatattt tgggcttctt tttttgttta aaactgtaaa tttgaatttt atttgaaaaa 60

attttggcta gaatttgaaa attccctttg aaaaaataaa acatcatagt attataatac 120

cgataatcaa attggtttatt ttgatatgaa ggagattaaa atg gaa ata aag aaa 175

Met Glu Ile Lys Lys

1

5

cat ttt aag ttg tac aaa agc ggc aaa caa tgg gta aca gca gca gtg	223
His Phe Lys Leu Tyr Lys Ser Gly Lys Gln Trp Val Thr Ala Ala Val	
10 15 20	
gct acc gtt gcc gtt tca act gcg ctt ctt tac gga gga gtt gcg cat	271
Ala Thr Val Ala Val Ser Thr Ala Leu Leu Tyr Gly Gly Val Ala His	
25 30 35	
gct gac caa caa gtt cag caa gct tcc acg act caa gac caa act tct	319
Ala Asp Gln Gln Val Gln Gln Ala Ser Thr Thr Gln Asp Gln Thr Ser	
40 45 50	
acc gta aat aat gat act gat aaa aca gta gct tta gat act aat act	367
Thr Val Asn Asn Asp Thr Asp Lys Thr Val Ala Leu Asp Thr Asn Thr	
55 60 65	
gac cag tca gct caa aca act gat aaa aaa caa gta gta tca aat act	415
Asp Gln Ser Ala Gln Thr Thr Asp Lys Lys Gln Val Val Ser Asn Thr	
70 75 80 85	
aac caa agc ⁵ aaa act gat gac act tca aca gct gat aag aat tct act	463
Asn Gln Ser Lys Thr Asp Asp Thr Ser Thr Ala Asp Lys Asn Ser Thr	
90 95 100	
tct aca cct gtt tct gtt ttg cca tct aat aat act gaa aaa caa gct	511
Ser Thr Pro Val Ser Val Leu Pro Ser Asn Asn Thr Glu Lys Gln Ala	
105 110 115	
aaa aat tat aat gag caa gac aaa gga aac tat ggg aat att gat act	559
Lys Asn Tyr Asn Glu Gln Asp Lys Gly Asn Tyr Gly Asn Ile Asp Thr	
120 125 130	
gct tac ttt agc aat aat caa ttg cat gtt tca gga tgg aat gca acg	607
Ala Tyr Phe Ser Asn Asn Gln Leu His Val Ser Gly Trp Asn Ala Thr	
135 140 145	
aac gca tct caa gga aca aac agt cga caa atc att gtg cgt gat atc	655
Asn Ala Ser Gln Gly Thr Asn Ser Arg Gln Ile Ile Val Arg Asp Ile	
150 155 160 165	
aca acc aat aat gaa tta ggt cgc act gat gta aca aac aat gtt gca	703
Thr Thr Asn Asn Glu Leu Gly Arg Thr Asp Val Thr Asn Asn Val Ala	
170 175 180	
cgc cca gac gtt aag aat gtt cat aat gtt tat aac gct gat aat tct	751
Arg Pro Asp Val Lys Asn Val His Asn Val Tyr Asn Ala Asp Asn Ser	
185 190 195	

gga ttt gat gtt aat gtc aat att gac ttt agc aag atg aaa gat tat	799
Gly Phe Asp Val Asn Val Asn Ile Asp Phe Ser Lys Met Lys Asp Tyr	
200 205 210	
cgg gat tca att gaa att gtt agt cga tac agt gga aac ggt aaa tct	847
Arg Asp Ser Ile Glu Ile Val Ser Arg Tyr Ser Gly Asn Gly Lys Ser	
215 220 225	
gtt gac tgg tgg tcc caa ccg atc act ttt gac aaa aac aac tat gct	895
Val Asp Trp Trp Ser Gln Pro Ile Thr Phe Asp Lys Asn Asn Tyr Ala	
230 235 240 245	
tat ctt gat aca ttt gaa gtg aaa aat ggc gaa tta cat gca acc gga	943
Tyr Leu Asp Thr Phe Glu Val Lys Asn Gly Glu Leu His Ala Thr Gly	
250 255 260	
tgg aat gct act aat agt gcg att aac tat aat cac cat ttt gtg att	991
Trp Asn Ala Thr Asn Ser Ala Ile Asn Tyr Asn His His Phe Val Ile	
265 270 275	
ttg ttt gat caa acg aat ggt aaa gaa gta gca cga caa gaa gtt cgt	1039
Leu Phe Asp Gln Thr Asn Gly Lys Glu Val Ala Arg Gln Glu Val Arg	
280 285 290	
gaa ggt caa tca cgc cca gat gtt gct aag gta tat cca caa gta gtt	1087
Glu Gly Gln Ser Arg Pro Asp Val Ala Lys Val Tyr Pro Gln Val Val	
295 300 305	
ggt gct gcc aac tca ggc ttt aat gtg aca ttt aat atc agt gat tta	1135
Gly Ala Ala Asn Ser Gly Phe Asn Val Thr Phe Asn Ile Ser Asp Leu	
310 315 320 325	
gat tat act cac cag tac caa gtt ctt agt cgt tac agc aat tct gat	1183
Asp Tyr Thr His Gln Tyr Gln Val Leu Ser Arg Tyr Ser Asn Ser Asp	
330 335 340	
aat ggc gaa ggt gat aac gtt acc tac tgg ttt aat cca caa tcc att	1231
Asn Gly Glu Gly Asp Asn Val Thr Tyr Trp Phe Asn Pro Gln Ser Ile	
345 350 355	
gct cct gct aat caa agt aac cag ggt tat cta gac tca ttt gat att	1279
Ala Pro Ala Asn Gln Ser Asn Gln Gly Tyr Leu Asp Ser Phe Asp Ile	
360 365 370	
agt aaa aat ggt gaa gta aca gta act gga tgg aac gct act gac ttg	1327
Ser Lys Asn Gly Glu Val Thr Val Thr Gly Trp Asn Ala Thr Asp Leu	
375 380 385	

tca gaa tta caa aac aac cat tat gtg att cta ttt gat cag aca gca	1375
Ser Glu Leu Gln Asn Asn His Tyr Val Ile Leu Phe Asp Gln Thr Ala	
390 395 400 405	
ggc aaa caa gtt gca tct gct aaa gct gat tta att tca cgt cca gat	1423
Gly Lys Gln Val Ala Ser Ala Lys Ala Asp Leu Ile Ser Arg Pro Asp	
410 415 420	
gtt gct aaa gct tat cca aca gta aaa aca gct aca aat tct ggc ttc	1471
Val Ala Lys Ala Tyr Pro Thr Val Lys Thr Ala Thr Asn Ser Gly Phe	
425 430 435	
aag gta aca ttt aag gtt aat aac tta caa ccg ggt cac caa tac agc	1519
Lys Val Thr Phe Lys Val Asn Asn Leu Gln Pro Gly His Gln Tyr Ser	
440 445 450	
gtt gta agt cgt ttc tct gcc gat gaa aat ggt aat ggt aat gat aag	1567
Val Val Ser Arg Phe Ser Ala Asp Glu Asn Gly Asn Gly Asn Asp Lys	
455 460 465	
cgc cat aca gat tac tgg ttt agt cca gta ata tta aac cag act gct	1615
Arg His Thr Asp Tyr Trp Phe Ser Pro Val Ile Leu Asn Gln Thr Ala	
470 475 480 485	
tca aac att gat act att aca atg aca tct aat ggt tta cat att gca	1663
Ser Asn Ile Asp Thr Ile Thr Met Thr Ser Asn Gly Leu His Ile Ala	
490 495 500	
ggg tgg atg gca agt gat aac tca att aat gaa aca act cca tac gct	1711
Gly Trp Met Ala Ser Asp Asn Ser Ile Asn Glu Thr Thr Pro Tyr Ala	
505 510 515	
att atc ctc aat aat gga aaa gaa gtt act cgt caa aag atg agc tta	1759
Ile Ile Leu Asn Asn Gly Lys Glu Val Thr Arg Gln Lys Met Ser Leu	
520 525 530	
acc gcc cgt cca gat gta gca gca gta tat cct tca ctt tat aat agt	1807
Thr Ala Arg Pro Asp Val Ala Ala Val Tyr Pro Ser Leu Tyr Asn Ser	
535 540 545	
gct gtt agt ggt ttt gac act act att aaa ttg act aat gat caa tat	1855
Ala Val Ser Gly Phe Asp Thr Thr Ile Lys Leu Thr Asn Asp Gln Tyr	
550 555 560 565	
caa gcg ctt aat ggc caa tta caa gta ttg tta cgt ttt tct aaa gct	1903
Gln Ala Leu Asn Gly Gln Leu Gln Val Leu Leu Arg Phe Ser Lys Ala	
570 575 580	

gct gat ggt aat cca agt ggt gat aat act gta act gat caa ttt agt	1951
Ala Asp Gly Asn Pro Ser Gly Asp Asn Thr Val Thr Asp Gln Phe Ser	
585 590 595	
aaa aat tat gca act act ggt gga aac ttt gac tat gta aaa gta aat	1999
Lys Asn Tyr Ala Thr Thr Gly Gly Asn Phe Asp Tyr Val Lys Val Asn	
600 605 610	
ggt aat caa gtt gaa ttt agt ggt tgg cac gca act aac caa tca aat	2047
Gly Asn Gln Val Glu Phe Ser Gly Trp His Ala Thr Asn Gln Ser Asn	
615 620 625	
gat aaa gat tca caa tgg att att gtt tta gtt aat ggt aag gaa gta	2095
Asp Lys Asp Ser Gln Trp Ile Ile Val Leu Val Asn Gly Lys Glu Val	
630 635 640 645	
aag cgt caa tta gtt aat gat act aaa gag gga gct gct ggc ttc aac	2143
Lys Arg Gln Leu Val Asn Asp Thr Lys Glu Gly Ala Ala Gly Phe Asn	
650 655 660	
cga aac gat gtc tac aaa gta aat cca gct att gaa aac agt tct atg	2191
Arg Asn Asp Val Tyr Lys Val Asn Pro Ala Ile Glu Asn Ser Ser Met	
665 670 675	
tct gga ttc caa ggc att att act tta cct gtg aca gtt aaa aac gaa	2239
Ser Gly Phe Gln Gly Ile Ile Thr Leu Pro Val Thr Val Lys Asn Glu	
680 685 690	
aat gtc caa ctt gtt cat cgg ttt agt aac gat gtg aag act ggt gaa	2287
Asn Val Gln Leu Val His Arg Phe Ser Asn Asp Val Lys Thr Gly Glu	
695 700 705	
ggt aac tat gtt gat ttc tgg tca gag cta atg cct gtt aag gat agc	2335
Gly Asn Tyr Val Asp Phe Trp Ser Glu Leu Met Pro Val Lys Asp Ser	
710 715 720 725	
ttc caa aag ggg aat ggc cca ctt aag caa ttt ggc tta caa act att	2383
Phe Gln Lys Gly Asn Gly Pro Leu Lys Gln Phe Gly Leu Gln Thr Ile	
730 735 740	
aac ggt caa caa tat tat att gac cca aca act ggt caa cca cgt aag	2431
Asn Gly Gln Gln Tyr Tyr Ile Asp Pro Thr Thr Gly Gln Pro Arg Lys	
745 750 755	
aat ttc tta tta caa agt gga aat aat tgg att tac ttt gat agt gat	2479
Asn Phe Leu Leu Gln Ser Gly Asn Asn Trp Ile Tyr Phe Asp Ser Asp	
760 765 770	

act ggt gtg ggt act aat gca ctt gaa tta caa ttt gca aag gga act	2527
Thr Gly Val Gly Thr Asn Ala Leu Glu Leu Gln Phe Ala Lys Gly Thr	
775 780 785	
gtt tca tct aat gaa caa tac cgt aac ggt aat gca gct tac agt tat	2575
Val Ser Ser Asn Glu Gln Tyr Arg Asn Gly Asn Ala Ala Tyr Ser Tyr	
790 795 800 805	
gat gac aag agt atc gaa aat gta aat ggt tac tta aca gca gat aca	2623
Asp Asp Lys Ser Ile Glu Asn Val Asn Gly Tyr Leu Thr Ala Asp Thr	
810 815 820	
tgg tac cgt cca aaa cag atc tta aag gat gga act acc tgg act gac	2671
Trp Tyr Arg Pro Lys Gln Ile Leu Lys Asp Gly Thr Thr Trp Thr Asp	
825 830 835	
tca aaa gaa aca gat atg cga cca atc ttg atg gta tgg tgg cct aat	2719
Ser Lys Glu Thr Asp Met Arg Pro Ile Leu Met Val Trp Trp Pro Asn	
840 845 850	
act ctt acc caa gca tac tac ctt aat tac atg aaa caa cat ggt aat	2767
Thr Leu Thr Gln Ala Tyr Tyr Leu Asn Tyr Met Lys Gln His Gly Asn	
855 860 865	
tta tta cca tct gct tta cca ttc ttt aat gcg gat gct gat cct gca	2815
Leu Leu Pro Ser Ala Leu Pro Phe Phe Asn Ala Asp Ala Asp Pro Ala	
870 875 880 885	
gaa tta aat cat tat tcc gaa att gtg caa caa aat att gaa aaa cga	2863
Glu Leu Asn His Tyr Ser Glu Ile Val Gln Gln Asn Ile Glu Lys Arg	
890 895 900	
att agt gaa acc gga aat act gat tgg tta cgt act tta atg cac gat	2911
Ile Ser Glu Thr Gly Asn Thr Asp Trp Leu Arg Thr Leu Met His Asp	
905 910 915	
ttt gtt act aac aat ccg atg tgg aat aag gat agt gaa aat gtt aac	2959
Phe Val Thr Asn Asn Pro Met Trp Asn Lys Asp Ser Glu Asn Val Asn	
920 925 930	
ttt agt ggt att caa ttc caa ggc gga ttc tta aag tat gaa aac tca	3007
Phe Ser Gly Ile Gln Phe Gln Gly Gly Phe Leu Lys Tyr Glu Asn Ser	
935 940 945	
gat tta acg cct tat gct aac tct gat tat cgc tta ctt ggt cgg atg	3055
Asp Leu Thr Pro Tyr Ala Asn Ser Asp Tyr Arg Leu Leu Gly Arg Met	
950 955 960 965	

cca atc aat att aag gat caa aca tat cgg gga caa gaa ttc cta ctt	3103
Pro Ile Asn Ile Lys Asp Gln Thr Tyr Arg Gly Gln Glu Phe Leu Leu	
970 975 980	
gct aac gat att gat aac tct aat cct gtt gtt caa gca gaa caa tta	3151
Ala Asn Asp Ile Asp Asn Ser Asn Pro Val Val Gln Ala Glu Gln Leu	
985 990 995	
aac tgg tta tac tat ctc ttg aac ttt gga acg atc aca gct aat aat	3199
Asn Trp Leu Tyr Tyr Leu Leu Asn Phe Gly Thr Ile Thr Ala Asn Asn	
1000 1005 1010	
gat caa gct aat ttt gat tct gta cgg gta gat gca ccg gat aat att	3247
Asp Gln Ala Asn Phe Asp Ser Val Arg Val Asp Ala Pro Asp Asn Ile	
1015 1020 1025	
gat gcc gat ctt atg aat atc gct cag gac tac ttt aat gct gca tat	3295
Asp Ala Asp Leu Met Asn Ile Ala Gln Asp Tyr Phe Asn Ala Ala Tyr	
1030 1035 1040 1045	
ggt atg gac tca gat gct gtc tca aat aag cat att aat att ctt gaa	3343
Gly Met Asp Ser Asp Ala Val Ser Asn Lys His Ile Asn Ile Leu Glu	
1050 1055 1060	
gac tgg aat cat gct gat ccg gaa tac ttt aat aag atc gga aat cca	3391
Asp Trp Asn His Ala Asp Pro Glu Tyr Phe Asn Lys Ile Gly Asn Pro	
1065 1070 1075	
caa ttg aca atg gat gat act att aag aat tcc ctg aat cat ggg ctt	3439
Gln Leu Thr Met Asp Asp Thr Ile Lys Asn Ser Leu Asn His Gly Leu	
1080 1085 1090	
tca gat gca act aat cgt tgg gga tta gat gca att gtt cat cag tca	3487
Ser Asp Ala Thr Asn Arg Trp Gly Leu Asp Ala Ile Val His Gln Ser	
1095 1100 1105	
tta gct gat cgt gaa aat aat tcc acg gaa aat gtt gta att cct aat	3535
Leu Ala Asp Arg Glu Asn Asn Ser Thr Glu Asn Val Val Ile Pro Asn	
1110 1115 1120 1125	
tac agt ttc gtt cgg gct cac gat aat aat tct caa gat caa att caa	3583
Tyr Ser Phe Val Arg Ala His Asp Asn Asn Ser Gln Asp Gln Ile Gln	
1130 1135 1140	
aat gct att cgt gat gta aca ggc aaa gat tac cat act ttc act ttt	3631
Asn Ala Ile Arg Asp Val Thr Gly Lys Asp Tyr His Thr Phe Thr Phe	
1145 1150 1155	

gaa gat gag caa aag ggt att gat gcg tac att caa gat caa aat tca	3679
Glu Asp Glu Gln Lys Gly Ile Asp Ala Tyr Ile Gln Asp Gln Asn Ser	
1160 1165 1170	
aca gtg aag aaa tat aac ctt tat aat att ccg gct tca tac gca att	3727
Thr Val Lys Lys Tyr Asn Leu Tyr Asn Ile Pro Ala Ser Tyr Ala Ile	
1175 1180 1185	
ctt tta act aac aag gat aca att cca cgt gta tac tat ggt gac ttg	3775
Leu Leu Thr Asn Lys Asp Thr Ile Pro Arg Val Tyr Tyr Gly Asp Leu	
1190 1195 1200 1205	
tat act gat ggt ggc caa tac atg gaa cat caa aca cgt tac tat gat	3823
Tyr Thr Asp Gly Gly Gln Tyr Met Glu His Gln Thr Arg Tyr Tyr Asp	
1210 1215 1220	
act tta acg aac ctg ctt aaa tca cga gtt aag tat gtt gcc ggt ggc	3871
Thr Leu Thr Asn Leu Leu Lys Ser Arg Val Lys Tyr Val Ala Gly Gly	
1225 1230 1235	
caa tca atg caa aca atg agc gtt ggc ggc aat aat aac att tta act	3919
Gln Ser Met Gln Thr Met Ser Val Gly Gly Asn Asn Asn Ile Leu Thr	
1240 1245 1250	
agt gtt cgt tat ggt aaa ggt gcg atg aca gct act gat act ggt act	3967
Ser Val Arg Tyr Gly Lys Gly Ala Met Thr Ala Thr Asp Thr Gly Thr	
1255 1260 1265	
gat gaa acc aga aca caa ggt att ggg gtt gtt gta agt aat acg cca	4015
Asp Glu Thr Arg Thr Gln Gly Ile Gly Val Val Val Ser Asn Thr Pro	
1270 1275 1280 1285	
aat cta aag cta ggt gtc aac gat aaa gta gtt ctt cat atg gga gct	4063
Asn Leu Lys Leu Gly Val Asn Asp Lys Val Val Leu His Met Gly Ala	
1290 1295 1300	
gcg cac aag aac caa caa tat cgg gca gcc gtg ttg acg aca act gat	4111
Ala His Lys Asn Gln Gln Tyr Arg Ala Ala Val Leu Thr Thr Thr Asp	
1305 1310 1315	
gga gtc att aat tat act tct gat caa ggg gca ccg gtt gca atg act	4159
Gly Val Ile Asn Tyr Thr Ser Asp Gln Gly Ala Pro Val Ala Met Thr	
1320 1325 1330	
gac gag aac ggt gat cta tac tta tct agt cat aac cta gtt gtt aat	4207
Asp Glu Asn Gly Asp Leu Tyr Leu Ser Ser His Asn Leu Val Val Asn	
1335 1340 1345	

ggc	aaa	gaa	gaa	gca	gat	aca	gct	gtt	caa	ggc	tat	gct	aac	cct	gat	4255
Gly	Lys	Glu	Glu	Ala	Asp	Thr	Ala	Val	Gln	Gly	Tyr	Ala	Asn	Pro	Asp	
1350					1355				1360					1365		
ggt	tca	gga	tat	ctt	gct	gta	tgg	gta	cca	gtt	gga	gca	agt	gat	aac	4303
Val	Ser	Gly	Tyr	Leu	Ala	Val	Trp	Val	Pro	Val	Gly	Ala	Ser	Asp	Asn	
				1370				1375						1380		
caa	gat	gct	cga	act	gct	cca	tct	act	gaa	aag	aat	agt	ggc	aac	tct	4351
Gln	Asp	Ala	Arg	Thr	Ala	Pro	Ser	Thr	Glu	Lys	Asn	Ser	Gly	Asn	Ser	
			1385				1390						1395			
gca	tac	aga	aca	aat	gct	gct	ttt	gat	tca	aat	gtt	att	ttt	gaa	gcc	4399
Ala	Tyr	Arg	Thr	Asn	Ala	Ala	Phe	Asp	Ser	Asn	Val	Ile	Phe	Glu	Ala	
			1400				1405						1410			
ttt	tct	aac	ttt	gtc	tat	aca	cca	aca	aag	gaa	agt	gaa	cgt	gct	aat	4447
Phe	Ser	Asn	Phe	Val	Tyr	Thr	Pro	Thr	Lys	Glu	Ser	Glu	Arg	Ala	Asn	
		1415				1420						1425				
gtt	cga	att	gcc	caa	aat	gct	gat	ttc	ttt	gct	tca	tta	ggc	ttt	act	4495
Val	Arg	Ile	Ala	Gln	Asn	Ala	Asp	Phe	Phe	Ala	Ser	Leu	Gly	Phe	Thr	
1430					1435					1440					1445	
tct	ttc	gag	atg	gcg	cca	caa	tat	aat	tca	agt	aaa	gat	cgc	aca	ttc	4543
Ser	Phe	Glu	Met	Ala	Pro	Gln	Tyr	Asn	Ser	Ser	Lys	Asp	Arg	Thr	Phe	
				1450					1455					1460		
cta	gat	tca	aca	att	gat	aac	gga	tat	gcg	ttt	act	gat	cgt	tat	gat	4591
Leu	Asp	Ser	Thr	Ile	Asp	Asn	Gly	Tyr	Ala	Phe	Thr	Asp	Arg	Tyr	Asp	
			1465					1470					1475			
ctt	gga	atg	agt	gag	cct	aat	aag	tac	gga	aca	gat	gaa	gat	cta	cgt	4639
Leu	Gly	Met	Ser	Glu	Pro	Asn	Lys	Tyr	Gly	Thr	Asp	Glu	Asp	Leu	Arg	
		1480					1485						1490			
aat	gcc	att	caa	gcg	ctc	cat	aaa	gct	ggc	tta	caa	gta	atg	gcg	gat	4687
Asn	Ala	Ile	Gln	Ala	Leu	His	Lys	Ala	Gly	Leu	Gln	Val	Met	Ala	Asp	
		1495				1500						1505				
tgg	gtt	cct	gac	caa	atc	tat	aac	ctt	cct	gga	aaa	gaa	gtt	gct	aca	4735
Trp	Val	Pro	Asp	Gln	Ile	Tyr	Asn	Leu	Pro	Gly	Lys	Glu	Val	Ala	Thr	
1510					1515					1520					1525	
gtc	act	cga	gta	gat	gat	cgt	ggc	aat	gta	tgg	aaa	gat	gct	atc	att	4783
Val	Thr	Arg	Val	Asp	Asp	Arg	Gly	Asn	Val	Trp	Lys	Asp	Ala	Ile	Ile	
				1530				1535					1540			

aat aat aat ctg tat gtt gtt aat act att ggt ggt ggc gaa tac cag	4831
Asn Asn Asn Leu Tyr Val Val Asn Thr Ile Gly Gly Gly Glu Tyr Gln	
1545 1550 1555	
aag aag tat ggt gga gca ttc ctc gat aag tta caa aaa ctt tat cct	4879
Lys Lys Tyr Gly Gly Ala Phe Leu Asp Lys Leu Gln Lys Leu Tyr Pro	
1560 1565 1570	
gaa atc ttc aca aag aag caa gtt tca act ggt gtt gct att gat cct	4927
Glu Ile Phe Thr Lys Lys Gln Val Ser Thr Gly Val Ala Ile Asp Pro	
1575 1580 1585	
tca caa aag ata act gaa tgg tca gca aaa tac ttt aat gga aca aac	4975
Ser Gln Lys Ile Thr Glu Trp Ser Ala Lys Tyr Phe Asn Gly Thr Asn	
1590 1595 1600 1605	
att ctc cat cgt ggt tct ggt tat gta cta aaa gct gat ggt ggt caa	5023
Ile Leu His Arg Gly Ser Gly Tyr Val Leu Lys Ala Asp Gly Gly Gln	
1610 1615 1620	
tac tac aac tta ggt act act aca aag caa ttc ttg cca att caa tta	5071
Tyr Tyr Asn Leu Gly Thr Thr Thr Lys Gln Phe Leu Pro Ile Gln Leu	
1625 1630 1635	
act ggt gaa aag aaa caa gga aat gaa ggc ttt gtt aag ggt aat gat	5119
Thr Gly Glu Lys Lys Gln Gly Asn Glu Gly Phe Val Lys Gly Asn Asp	
1640 1645 1650	
gga aat tac tac ttc tat gac tta gca ggt aat atg gtt aag aat acc	5167
Gly Asn Tyr Tyr Phe Tyr Asp Leu Ala Gly Asn Met Val Lys Asn Thr	
1655 1660 1665	
ttt att gaa gat agt gtt ggc aac tgg tac ttc ttt gac caa gat ggt	5215
Phe Ile Glu Asp Ser Val Gly Asn Trp Tyr Phe Phe Asp Gln Asp Gly	
1670 1675 1680 1685	
aag atg gtt gaa aat aaa cat ttc gtt gat gtt gat tct tat ggt gaa	5263
Lys Met Val Glu Asn Lys His Phe Val Asp Val Asp Ser Tyr Gly Glu	
1690 1695 1700	
aaa ggt act tac ttc ttc ttg aag aat ggt gta tca ttc cgt ggg gga	5311
Lys Gly Thr Tyr Phe Phe Leu Lys Asn Gly Val Ser Phe Arg Gly Gly	
1705 1710 1715	
tta gtg caa act gac aat ggt act tat tac ttt gat aat tat gga aag	5359
Leu Val Gln Thr Asp Asn Gly Thr Tyr Tyr Phe Asp Asn Tyr Gly Lys	
1720 1725 1730	

atg gta cgt aat caa act att aat gca ggt gcc atg att tat acc tta 5407
 Met Val Arg Asn Gln Thr Ile Asn Ala Gly Ala Met Ile Tyr Thr Leu
 1735 1740 1745

gat gaa aac ggt aag ctt ata aag gct agt tat aat tca gat gcc gaa 5455
 Asp Glu Asn Gly Lys Leu Ile Lys Ala Ser Tyr Asn Ser Asp Ala Glu
 1750 1755 1760 1765

tat cca act tca act gat gtt ggt aag atg ctt gat caa aat aaa cta 5503
 Tyr Pro Thr Ser Thr Asp Val Gly Lys Met Leu Asp Gln Asn Lys Leu
 1770 1775 1780

taa atggaaataa ttagctgatt tccgtttctt agaatcgaaa gatttaataa 5556

ctgggggttaa aacggcccta caaaatctga tattgatata gagatattat ttcctatatac 5616

aatatcagat ttttgctttt tataaaattg attgtgacta ataagaatcc ggaagataac 5676

gttggttgta tatcagtga ttaagcaac atgaattaat tgaagatgac ggcaatgatt 5736

aaaagtcggt ctgatgatta ttgatgtatt actagtattt gggtttttatc atttatatatt 5796

ttactgttat tgggtgtcata tattccacaa taacagtaaa ggtatatatg ctagttttatt 5856

ttttaagtaa ttataatatt ctgattataa tttggaaata ttcgctttta gcaaaaagggt 5916

agtaaacaga tcagaatcgt cattctgctt ttctactact aaaagtctgt tttaaattct 5976

aaactaaaaat aggctaaaca ctgatgttta tcatttatat ttttactgtt 6026

<210> 2

<211> 1781

<212> PRT

<213> Lactobacillus reuteri

<400> 2

Met Glu Ile Lys Lys His Phe Lys Leu Tyr Lys Ser Gly Lys Gln Trp
 1 5 10 15

Val Thr Ala Ala Val Ala Thr Val Ala Val Ser Thr Ala Leu Leu Tyr
 20 25 30

Gly Gly Val Ala His Ala Asp Gln Gln Val Gln Gln Ala Ser Thr Thr
 35 40 45

Gln Asp Gln Thr Ser Thr Val Asn Asn Asp Thr Asp Lys Thr Val Ala

50		55		60
Leu Asp Thr Asn Thr Asp Gln Ser Ala Gln Thr Thr Asp Lys Lys Gln				
65		70		75
Val Val Ser Asn Thr Asn Gln Ser Lys Thr Asp Asp Thr Ser Thr Ala				
	85		90	95
Asp Lys Asn Ser Thr Ser Thr Pro Val Ser Val Leu Pro Ser Asn Asn				
	100		105	110
Thr Glu Lys Gln Ala Lys Asn Tyr Asn Glu Gln Asp Lys Gly Asn Tyr				
	115		120	125
Gly Asn Ile Asp Thr Ala Tyr Phe Ser Asn Asn Gln Leu His Val Ser				
	130		135	140
Gly Trp Asn Ala Thr Asn Ala Ser Gln Gly Thr Asn Ser Arg Gln Ile				
145		150		155
Ile Val Arg Asp Ile Thr Thr Asn Asn Glu Leu Gly Arg Thr Asp Val				
	165		170	175
Thr Asn Asn Val Ala Arg Pro Asp Val Lys Asn Val His Asn Val Tyr				
	180		185	190
Asn Ala Asp Asn Ser Gly Phe Asp Val Asn Val Asn Ile Asp Phe Ser				
	195		200	205
Lys Met Lys Asp Tyr Arg Asp Ser Ile Glu Ile Val Ser Arg Tyr Ser				
	210		215	220
Gly Asn Gly Lys Ser Val Asp Trp Trp Ser Gln Pro Ile Thr Phe Asp				
225		230		235
Lys Asn Asn Tyr Ala Tyr Leu Asp Thr Phe Glu Val Lys Asn Gly Glu				
	245		250	255
Leu His Ala Thr Gly Trp Asn Ala Thr Asn Ser Ala Ile Asn Tyr Asn				
	260		265	270
His His Phe Val Ile Leu Phe Asp Gln Thr Asn Gly Lys Glu Val Ala				
	275		280	285
Arg Gln Glu Val Arg Glu Gly Gln Ser Arg Pro Asp Val Ala Lys Val				
	290		295	300
Tyr Pro Gln Val Val Gly Ala Ala Asn Ser Gly Phe Asn Val Thr Phe				

305		310		315		320
Asn Ile Ser Asp Leu Asp Tyr Thr His Gln Tyr Gln Val Leu Ser Arg						
	325		330			335
Tyr Ser Asn Ser Asp Asn Gly Glu Gly Asp Asn Val Thr Tyr Trp Phe						
	340		345			350
Asn Pro Gln Ser Ile Ala Pro Ala Asn Gln Ser Asn Gln Gly Tyr Leu						
	355		360			365
Asp Ser Phe Asp Ile Ser Lys Asn Gly Glu Val Thr Val Thr Gly Trp						
	370		375			380
Asn Ala Thr Asp Leu Ser Glu Leu Gln Asn Asn His Tyr Val Ile Leu						
	385		390			400
Phe Asp Gln Thr Ala Gly Lys Gln Val Ala Ser Ala Lys Ala Asp Leu						
	405		410			415
Ile Ser Arg Pro Asp Val Ala Lys Ala Tyr Pro Thr Val Lys Thr Ala						
	420		425			430
Thr Asn Ser Gly Phe Lys Val Thr Phe Lys Val Asn Asn Leu Gln Pro						
	435		440			445
Gly His Gln Tyr Ser Val Val Ser Arg Phe Ser Ala Asp Glu Asn Gly						
	450		455			460
Asn Gly Asn Asp Lys Arg His Thr Asp Tyr Trp Phe Ser Pro Val Ile						
	465		470			480
Leu Asn Gln Thr Ala Ser Asn Ile Asp Thr Ile Thr Met Thr Ser Asn						
	485		490			495
Gly Leu His Ile Ala Gly Trp Met Ala Ser Asp Asn Ser Ile Asn Glu						
	500		505			510
Thr Thr Pro Tyr Ala Ile Ile Leu Asn Asn Gly Lys Glu Val Thr Arg						
	515		520			525
Gln Lys Met Ser Leu Thr Ala Arg Pro Asp Val Ala Ala Val Tyr Pro						
	530		535			540
Ser Leu Tyr Asn Ser Ala Val Ser Gly Phe Asp Thr Thr Ile Lys Leu						
	545		550			560
Thr Asn Asp Gln Tyr Gln Ala Leu Asn Gly Gln Leu Gln Val Leu Leu						

[illegible]

820					825					830						
Thr	Thr	Trp	Thr	Asp	Ser	Lys	Glu	Thr	Asp	Met	Arg	Pro	Ile	Leu	Met	
835					840					845						
Val	Trp	Trp	Pro	Asn	Thr	Leu	Thr	Gln	Ala	Tyr	Tyr	Leu	Asn	Tyr	Met	
850					855					860						
Lys	Gln	His	Gly	Asn	Leu	Leu	Pro	Ser	Ala	Leu	Pro	Phe	Phe	Asn	Ala	
865					870					875					880	
Asp	Ala	Asp	Pro	Ala	Glu	Leu	Asn	His	Tyr	Ser	Glu	Ile	Val	Gln	Gln	
885					890					895						
Asn	Ile	Glu	Lys	Arg	Ile	Ser	Glu	Thr	Gly	Asn	Thr	Asp	Trp	Leu	Arg	
900					905					910						
Thr	Leu	Met	His	Asp	Phe	Val	Thr	Asn	Asn	Pro	Met	Trp	Asn	Lys	Asp	
915					920					925						
Ser	Glu	Asn	Val	Asn	Phe	Ser	Gly	Ile	Gln	Phe	Gln	Gly	Gly	Phe	Leu	
930					935					940						
Lys	Tyr	Glu	Asn	Ser	Asp	Leu	Thr	Pro	Tyr	Ala	Asn	Ser	Asp	Tyr	Arg	
945					950					955					960	
Leu	Leu	Gly	Arg	Met	Pro	Ile	Asn	Ile	Lys	Asp	Gln	Thr	Tyr	Arg	Gly	
965					970					975						
Gln	Glu	Phe	Leu	Leu	Ala	Asn	Asp	Ile	Asp	Asn	Ser	Asn	Pro	Val	Val	
980					985					990						
Gln	Ala	Glu	Gln	Leu	Asn	Trp	Leu	Tyr	Tyr	Leu	Leu	Asn	Phe	Gly	Thr	
995					1000					1005						
Ile	Thr	Ala	Asn	Asn	Asp	Gln	Ala	Asn	Phe	Asp	Ser	Val	Arg	Val	Asp	
1010					1015					1020						
Ala	Pro	Asp	Asn	Ile	Asp	Ala	Asp	Leu	Met	Asn	Ile	Ala	Gln	Asp	Tyr	
1025					1030					1035					1040	
Phe	Asn	Ala	Ala	Tyr	Gly	Met	Asp	Ser	Asp	Ala	Val	Ser	Asn	Lys	His	
1045					1050					1055						
Ile	Asn	Ile	Leu	Glu	Asp	Trp	Asn	His	Ala	Asp	Pro	Glu	Tyr	Phe	Asn	
1060					1065					1070						
Lys	Ile	Gly	Asn	Pro	Gln	Leu	Thr	Met	Asp	Asp	Thr	Ile	Lys	Asn	Ser	

1075	1080	1085
Leu Asn His Gly Leu Ser Asp Ala Thr Asn Arg Trp Gly Leu Asp Ala		
1090	1095	1100
Ile Val His Gln Ser Leu Ala Asp Arg Glu Asn Asn Ser Thr Glu Asn		
1105	1110	1115 1120
Val Val Ile Pro Asn Tyr Ser Phe Val Arg Ala His Asp Asn Asn Ser		
	1125	1130 1135
Gln Asp Gln Ile Gln Asn Ala Ile Arg Asp Val Thr Gly Lys Asp Tyr		
	1140	1145 1150
His Thr Phe Thr Phe Glu Asp Glu Gln Lys Gly Ile Asp Ala Tyr Ile		
	1155	1160 1165
Gln Asp Gln Asn Ser Thr Val Lys Lys Tyr Asn Leu Tyr Asn Ile Pro		
	1170	1175 1180
Ala Ser Tyr Ala Ile Leu Leu Thr Asn Lys Asp Thr Ile Pro Arg Val		
1185	1190	1195 1200
Tyr Tyr Gly Asp Leu Tyr Thr Asp Gly Gly Gln Tyr Met Glu His Gln		
	1205	1210 1215
Thr Arg Tyr Tyr Asp Thr Leu Thr Asn Leu Leu Lys Ser Arg Val Lys		
	1220	1225 1230
Tyr Val Ala Gly Gly Gln Ser Met Gln Thr Met Ser Val Gly Gly Asn		
	1235	1240 1245
Asn Asn Ile Leu Thr Ser Val Arg Tyr Gly Lys Gly Ala Met Thr Ala		
	1250	1255 1260
Thr Asp Thr Gly Thr Asp Glu Thr Arg Thr Gln Gly Ile Gly Val Val		
1265	1270	1275 1280
Val Ser Asn Thr Pro Asn Leu Lys Leu Gly Val Asn Asp Lys Val Val		
	1285	1290 1295
Leu His Met Gly Ala Ala His Lys Asn Gln Gln Tyr Arg Ala Ala Val		
	1300	1305 1310
Leu Thr Thr Thr Asp Gly Val Ile Asn Tyr Thr Ser Asp Gln Gly Ala		
	1315	1320 1325
Pro Val Ala Met Thr Asp Glu Asn Gly Asp Leu Tyr Leu Ser Ser His		

1330	1335	1340
Asn Leu Val Val Asn Gly Lys Glu Glu Ala Asp Thr Ala Val Gln Gly		
345	1350	1355 1360
Tyr Ala Asn Pro Asp Val Ser Gly Tyr Leu Ala Val Trp Val Pro Val		
1365	1370	1375
Gly Ala Ser Asp Asn Gln Asp Ala Arg Thr Ala Pro Ser Thr Glu Lys		
1380	1385	1390
Asn Ser Gly Asn Ser Ala Tyr Arg Thr Asn Ala Ala Phe Asp Ser Asn		
1395	1400	1405
Val Ile Phe Glu Ala Phe Ser Asn Phe Val Tyr Thr Pro Thr Lys Glu		
1410	1415	1420
Ser Glu Arg Ala Asn Val Arg Ile Ala Gln Asn Ala Asp Phe Phe Ala		
425	1430	1435 1440
Ser Leu Gly Phe Thr Ser Phe Glu Met Ala Pro Gln Tyr Asn Ser Ser		
1445	1450	1455
Lys Asp Arg Thr Phe Leu Asp Ser Thr Ile Asp Asn Gly Tyr Ala Phe		
1460	1465	1470
Thr Asp Arg Tyr Asp Leu Gly Met Ser Glu Pro Asn Lys Tyr Gly Thr		
1475	1480	1485
Asp Glu Asp Leu Arg Asn Ala Ile Gln Ala Leu His Lys Ala Gly Leu		
1490	1495	1500
Gln Val Met Ala Asp Trp Val Pro Asp Gln Ile Tyr Asn Leu Pro Gly		
505	1510	1515 1520
Lys Glu Val Ala Thr Val Thr Arg Val Asp Asp Arg Gly Asn Val Trp		
1525	1530	1535
Lys Asp Ala Ile Ile Asn Asn Asn Leu Tyr Val Val Asn Thr Ile Gly		
1540	1545	1550
Gly Gly Glu Tyr Gln Lys Lys Tyr Gly Gly Ala Phe Leu Asp Lys Leu		
1555	1560	1565
Gln Lys Leu Tyr Pro Glu Ile Phe Thr Lys Lys Gln Val Ser Thr Gly		
1570	1575	1580
Val Ala Ile Asp Pro Ser Gln Lys Ile Thr Glu Trp Ser Ala Lys Tyr		

585	1590	1595	1600
Phe Asn Gly Thr Asn Ile Leu His Arg Gly Ser Gly Tyr Val Leu Lys			
1605	1610	1615	
Ala Asp Gly Gly Gln Tyr Tyr Asn Leu Gly Thr Thr Thr Lys Gln Phe			
1620	1625	1630	
Leu Pro Ile Gln Leu Thr Gly Glu Lys Lys Gln Gly Asn Glu Gly Phe			
1635	1640	1645	
Val Lys Gly Asn Asp Gly Asn Tyr Tyr Phe Tyr Asp Leu Ala Gly Asn			
1650	1655	1660	
Met Val Lys Asn Thr Phe Ile Glu Asp Ser Val Gly Asn Trp Tyr Phe			
665	1670	1675	1680
Phe Asp Gln Asp Gly Lys Met Val Glu Asn Lys His Phe Val Asp Val			
1685	1690	1695	
Asp Ser Tyr Gly Glu Lys Gly Thr Tyr Phe Phe Leu Lys Asn Gly Val			
1700	1705	1710	
Ser Phe Arg Gly Gly Leu Val Gln Thr Asp Asn Gly Thr Tyr Tyr Phe			
1715	1720	1725	
Asp Asn Tyr Gly Lys Met Val Arg Asn Gln Thr Ile Asn Ala Gly Ala			
1730	1735	1740	
Met Ile Tyr Thr Leu Asp Glu Asn Gly Lys Leu Ile Lys Ala Ser Tyr			
745	1750	1755	1760
Asn Ser Asp Ala Glu Tyr Pro Thr Ser Thr Asp Val Gly Lys Met Leu			
1765	1770	1775	
Asp Gln Asn Lys Leu			
1780			

<210> 3
 <211> 13
 <212> PRT
 <213> Lactobacillus reuteri

<220>
 <221> REPEAT
 <222> (1)..(13)

<400> 3

Tyr Tyr Phe Tyr Asp Leu Ala Gly Asn Met Val Lys Asn
1 5 10

<210> 4

<211> 13

<212> PRT

<213> Lactobacillus reuteri

<220>

<221> REPEAT

<222> (1)..(13)

<400> 4

Trp Tyr Phe Phe Asp Gln Asp Gly Lys Met Val Glu Asn
1 5 10

<210> 5

<211> 13

<212> PRT

<213> Lactobacillus reuteri

<220>

<221> REPEAT

<222> (1)..(13)

<400> 5

Thr Tyr Tyr Phe Asp Asn Tyr Gly Lys Met Val Arg Asn
1 5 10

<210> 6

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Degenerate
primer GTFpr1

<400> 6

gayaakwsna aksynrtngt nsargc

26

<210> 7
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Degenerate
primer GTFpr2

<400> 7
gnkcncanattatccnctr na

22

<210> 8
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Degenerate
primer GTFpr3

<400> 8
acaaccacca tgggaattagg tcgcactgat gtaac

35

<210> 9
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Degenerate
primer GTFpr4

<400> 9
gccagctgga tccgtcgact agtttatttt tgatcaagca tcttacc

47

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/NL 01/00393

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/54 C12N9/10 C12N1/21 C12P19/18 C08B37/00
A23C9/123 //(C12N9/10, C12R1:225)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P C08B A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

CHEM ABS Data, BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, STRAND, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN GEEL-SCHUTTEN, G. H. ET AL: "Exopolysaccharide production by Lactobacillus reuteri, involving sucrase type of enzymes" MEDED. - FAC. LANDBOUWKD. TOEGEPASTE BIOL. WET. (UNIV. GENT), vol. 65, no. 3a, 2000, pages 197-201, XP000971140 the whole document --- -/-	1-16

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

29 August 2001

Date of mailing of the international search report

10/09/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

Internet Application No
PCT/NL 01/00393

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VAN GEEL-SCHUTTEN G H ET AL: "Biochemical and structural characterization of the glucan and fructan exopolysaccharides synthesized by the <i>Lactobacillus reuteri</i> wild-type strain and by mutant strains." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 65, no. 7, July 1999 (1999-07), pages 3008-3014, XP002154732 ISSN: 0099-2240 cited in the application	15,16
Y	abstract page 3010, left-hand column, last paragraph -right-hand column page 3013, left-hand column	1-14
Y	MONCHOIS VINCENT ET AL: "Cloning and sequencing of a gene coding for a novel dextransucrase from <i>Leuconostoc mesenteroides</i> NRRL B-1299 synthesizing only alpha(1-6) and alpha(1-3) linkages." GENE (AMSTERDAM), vol. 182, no. 1-2, 1996, pages 23-32, XP004071926 ISSN: 0378-1119 abstract figure 1; table 1	1-14
Y	SIMPSON CHRISTINE L ET AL: "Streptococcus salivarius ATCC 25975 possesses at least two genes coding for primer-independent glucosyltransferases." INFECTION AND IMMUNITY, vol. 63, no. 2, 1995, pages 609-621, XP002154733 ISSN: 0019-9567 the whole document	1-14
X	DATABASE EMBL 'Online! Accession U12643, 16 November 1995 (1995-11-16) VICKERMAN M M ET AL: "Streptococcus gordonii glucosyltransferase (gtfG), Dsg (dsg), RggD (rggD), and Y1bN-like hypothetical protein genes, complete cds; and hypothetical protein gene, partial cds." XP002176190 abstract	1,3,9-11

=D10

=D2